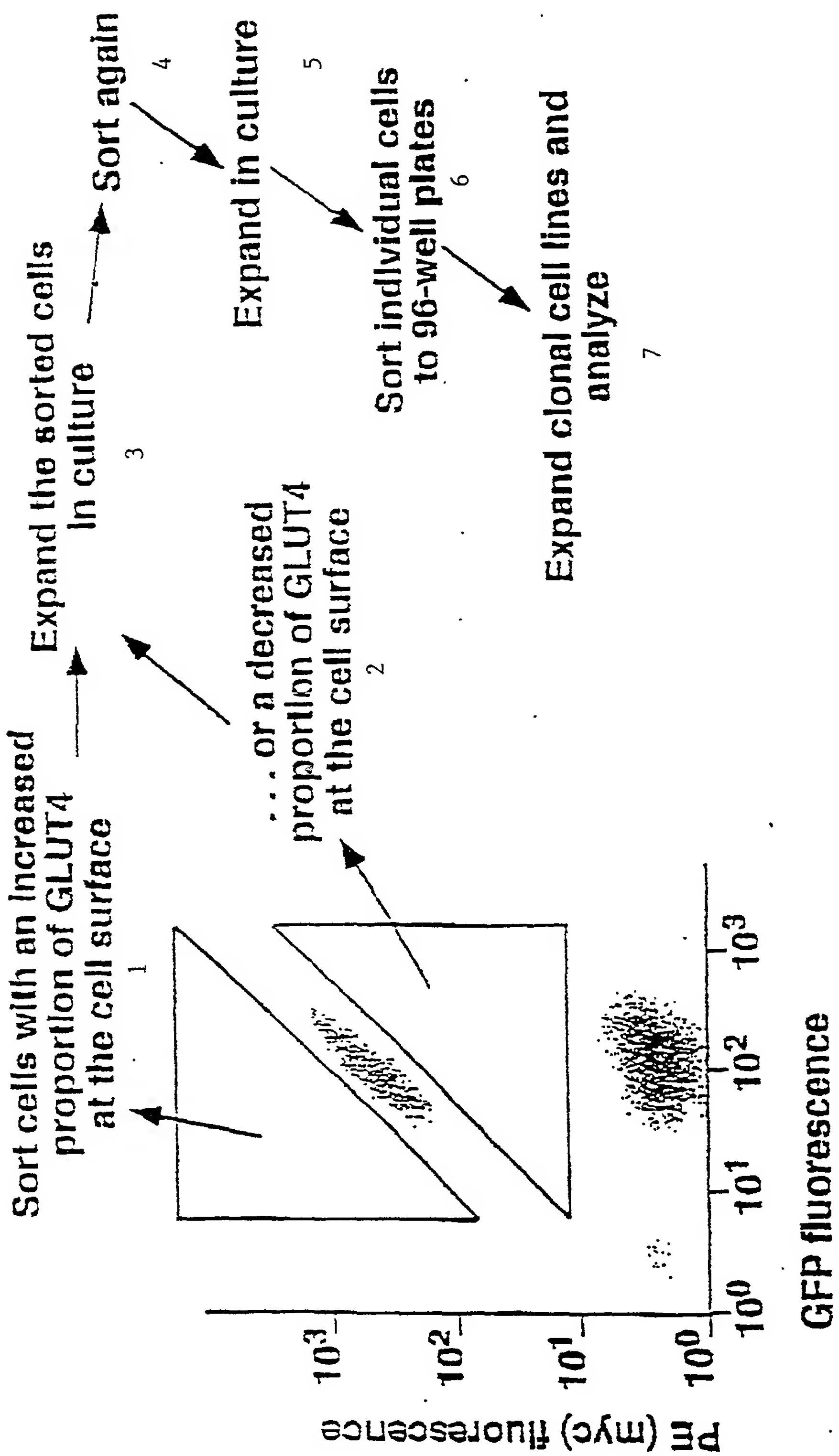

Sort cells with an increased proportion of GLUT4 at the cell surface
1
. . . or a decreased proportion of GLUT4 at the cell surface
2
Expand the sorted cells in culture
3
Sort again
4
Expand in culture
5
Sort individual cells to 96-well plates
6
Expand clonal cell lines and analyze
7
PE (myc) fluorescence
10^0
10^1
10^2
10^3
GFP fluorescence

Figure 1

Docket/App No.: 0399.2025-002
Title: Expression Cloning Method
Inventors:Jonathan S. Bogan, Harvey F. Lodish

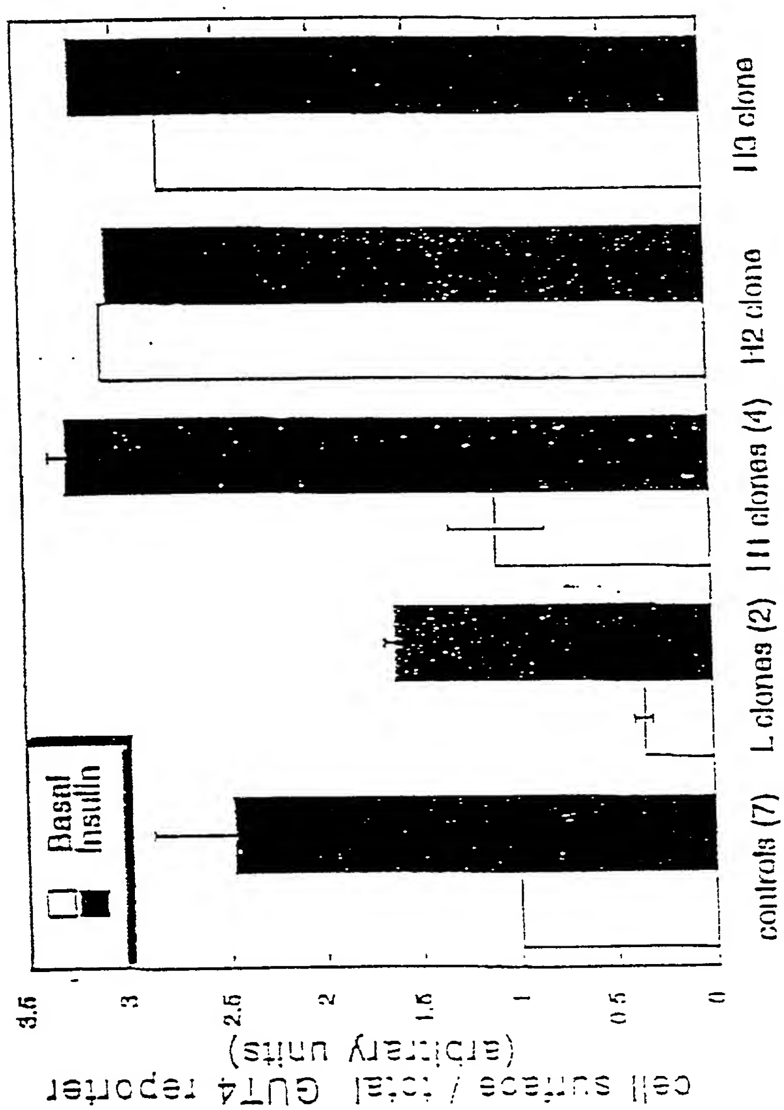


Figure 2

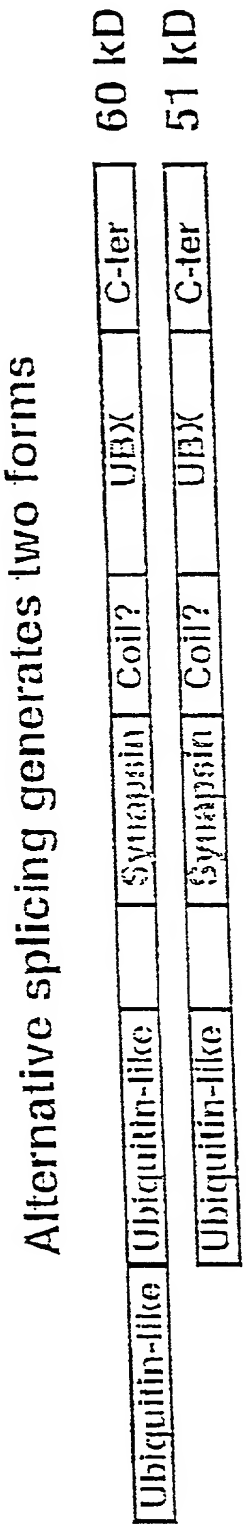

Alternative splicing generates two forms
Ubiquitin-like
Ubiquitin-like
Synapsin
Coil?
UBX
C-ter
60 kD
Ubiquitin-like
Synapsin
Coil?
UBX
C-ter
51 kD

Figure 3

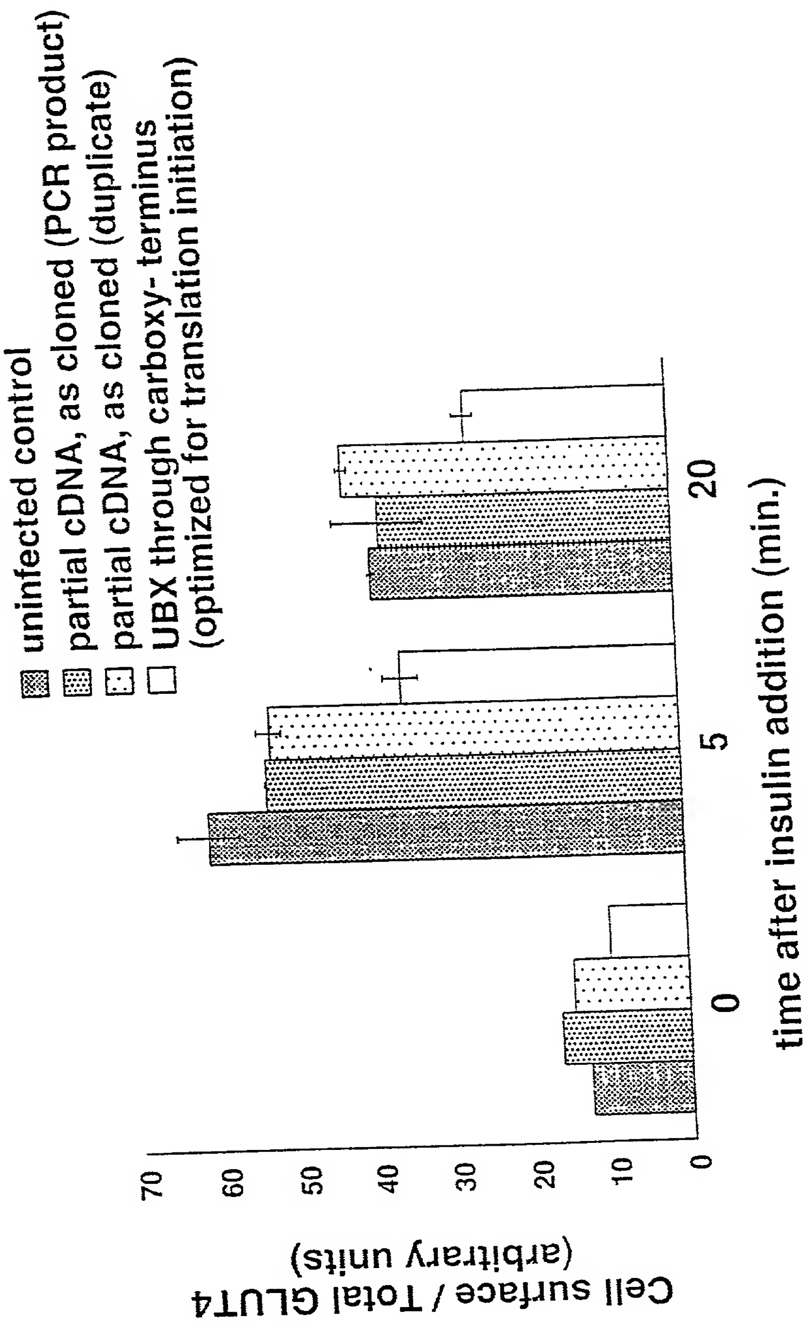

uninfected control
partial cDNA, as cloned (PCR product)
partial cDNA, as cloned (duplicate)
UBX through carboxy- terminus
(optimized for translation initiation)
Cell surface / Total GLUT4
(arbitrary units)
0
10
20
30
40
50
60
70
0
5
20
time after insulin addition (min.)

Figure 4

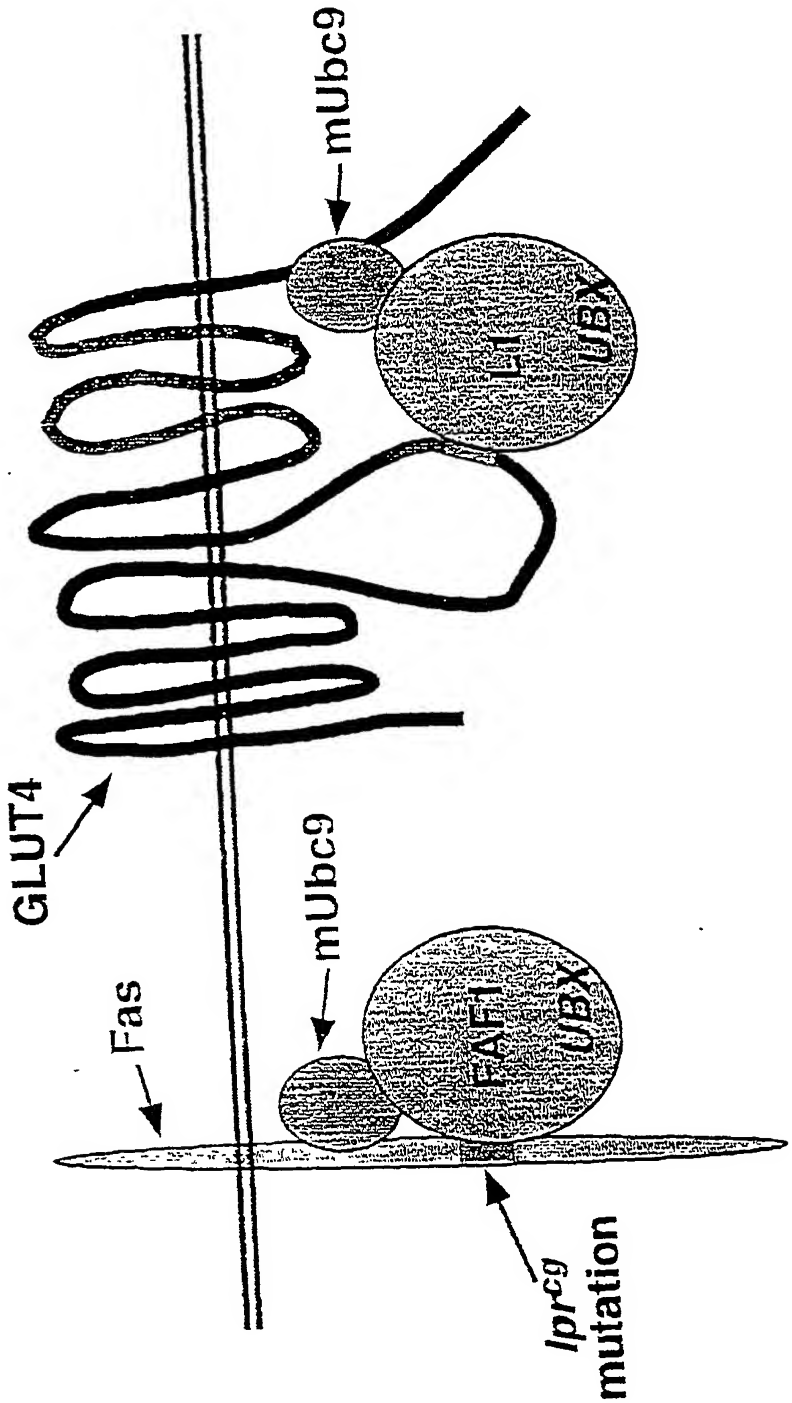


Figure 5

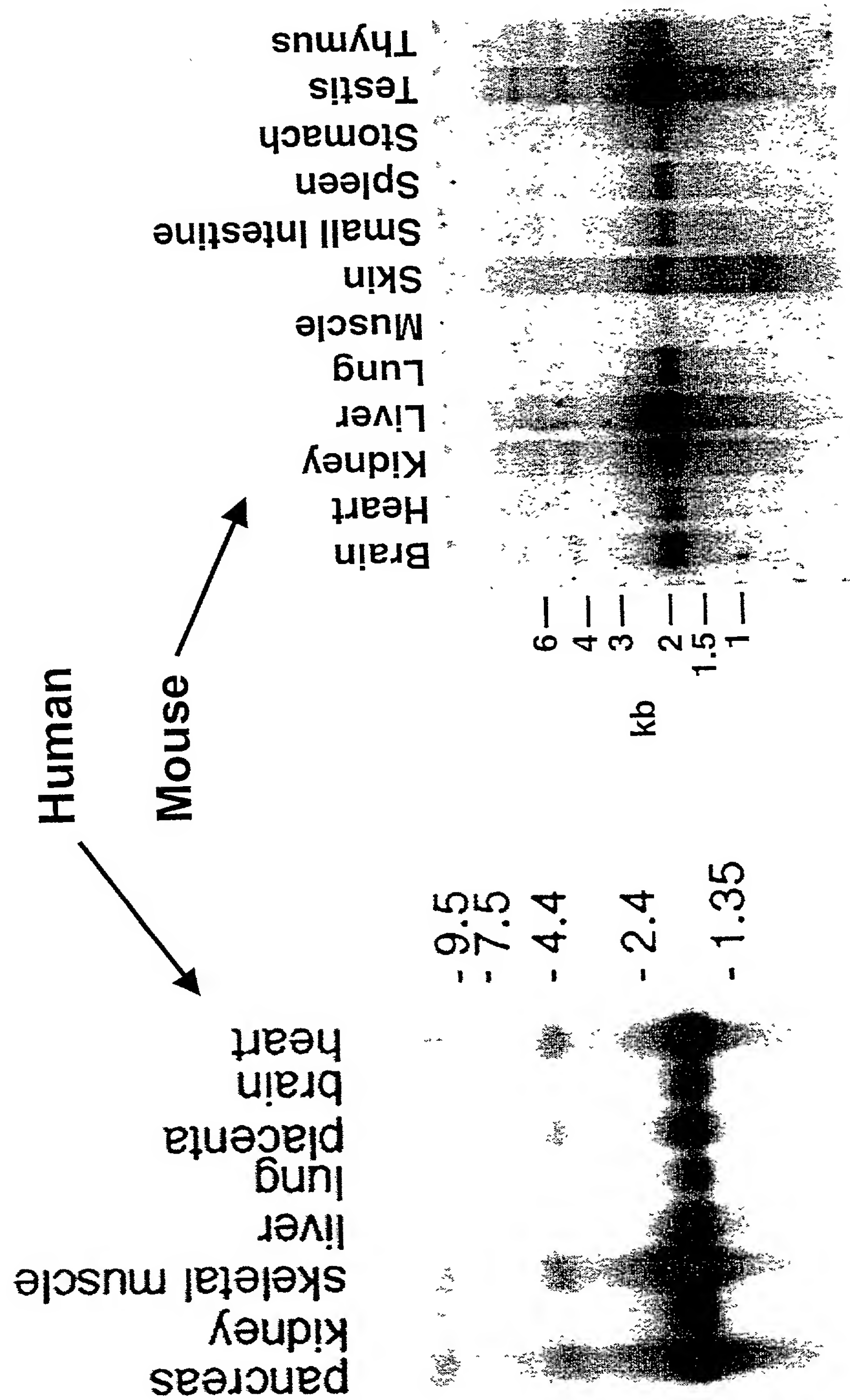


Figure 6B

Figure 6A

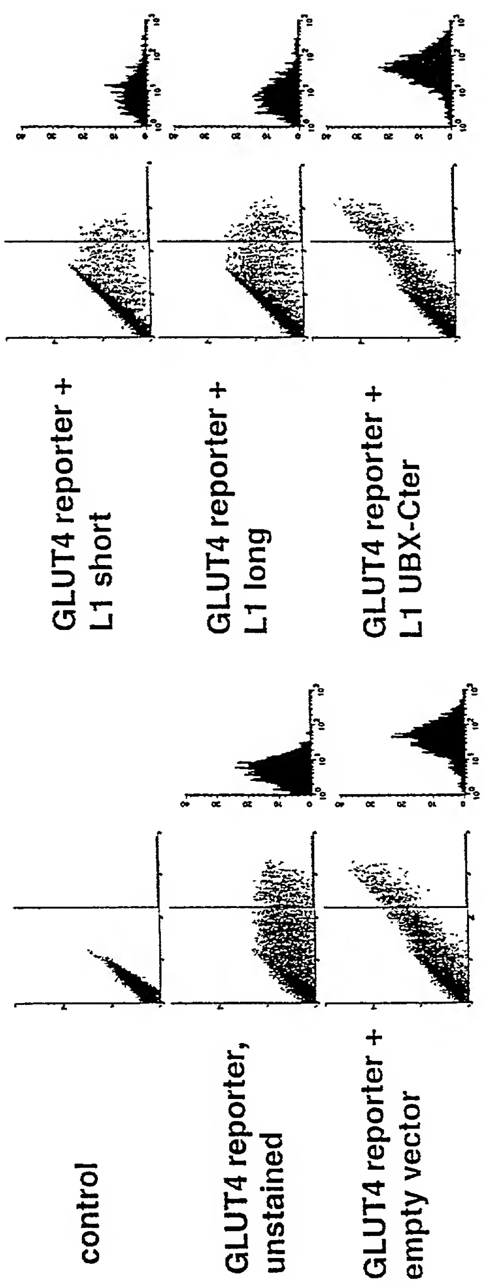

control
GLUT4 reporter, unstained
GLUT4 reporter + empty vector
GLUT4 reporter + L1 short
GLUT4 reporter + L1 long
GLUT4 reporter + L1 UBX-Cter

Figure 7

mmL1 long splice variant:
MAAPAGGGGSAVSVLAPNGRRHTVKVTPSTVLLQVLEDTCRRQDFNPSEY
DLKFQRTVLDLSLQWRFANLPNNAKLEMVPVSRSREGPENIVRIAFQLDD
GSRLQDAFCSRQTLWELLSHFAQTRERLQQLGEKTPVCVYMRNEVTGRAA
LQNTTLQSLGLTGGSATIRFVIKQCDTAGKQESIAVRSKAPGSPVSSLSA
DQASSSTLLPLNSGEFSRGDLNHEGDANTSGTGLEGGPKPTDAQTKQSTS
EPASAPFVPFSGGGQRLGGPSASLRPLTSPSANSSKSFSGPGGPSKPKKP
KPGEEPQQEPEPPVDRDPVVYHPDLEDLLQPWPAEVPDEFFEVTVDDVRR
RLAQLKSERKRLEEAPLVTKAFREAQMKEKLERYPKVALRVLFPDRYILQ
GFFRPSETVGDLRDFVRSHLGNPELSFYLFIAPPKMVLDDHTLTLFQANL
FPAALVHFGAEEPTGLYLEPGLLEHTVSPSTADVLVARCMSRASGSPPLL
PAPDPVSLESEPIAEDGALGPPEPIQGTAQPVKRSLGKVPKWLKLPASKR
*

Figure 8A

mmL1 short splice variant:
MVPVSRSREGPENIVRIAFQLDDGSRLQDAFCSRQTLWELLSHFAQTRER
LQQLGEKTPVCVYMRNEVTGRAALQNTTLQSLGLTGGSATIRFVIKQCDT
AGKQESIAVRSKAPGSPVSSLSADQASSSTLLPLNSGEFSRGDLNHEGDA
NTSGTGLEGGPKPTDAQTKQSTSEPASAPFVPFSGGGQRLGGPSASLRPL
TSPSANSSKSFSGPGGPSKPKKPKPGEEPQQEPEPPVDRDPVVYHPDLED
LLQPWPAEVPDEFFEVTVDDVRRRLAQLKSERKRLEEAPLVTKAFREAQM
KEKLERYPKVALRVLFPDRYILQGFFRPSETVGDLRDFVRSHLGNPELSF
YLFIAPPKMVLDDHTLTLFQANLFPAALVHFGAEEPTGLYLEPGLLEHTV
SPSTADVLVARCMSRASGSPPLLPAPDPVSLESEPIAEDGALGPPEPIQG
TAQPVKRSLGKVPKWLKLPASKR*

Figure 8B

hsL1 long splice variant:
MAAPAGGGGSAVSVLAPNGRRHTVKVTPSTVLLQVLEDTCRRQDFNPCEY
DLKFQRSVLDLSLQWRFANLPNNAKLEMVPASRSREGPENMVRIALQLDD
GSRLQDSFCSGQTLWELLSHFPQIRECLQHPGGATPVCVYTRDEVTGEAA
LRGTTLQSLGLTGGSATIRFVMKCYDPVGKTPGSLGSSASAGQAAASAPL
PLESGELSRGDLSRPEDADTSGPCCEHTQEKQSTRAPAAAPFVPFSGGGQ
RLGGPPGPTRPLTSSSAKLPKSLSSPGGPSKPKKSKSGQDPQQEQEQERE
RDPQQEQERERPVDREPVDREPVVCHPDLEERLQAWPAELPDEFFELTVD
DVRRRLAQLKSERKRLEEAPLVTKAFREAQIKEKLERYPKVALRVLFPDR
YVLQGFFRPSETVGDLRDFVRSHLGNPELSFYLFITPPKTVLDDHTQTLF
QANLFPAALVHLGAEEPAGVYLEPGLLEHAISPSAADVLVARYMSRAAGS
PSPLPAPDPAPKSEPAAEEGALVPPEPIPGTAQPVKRSLGKVPKWLKLPA
SKR*

Figure 8C

hsL1 short splice variant:
MVPASRSREGPENMVRIALQLDDGSRLQDSFCSGQTLWELLSHFPQIREC
LQHPGGATPVCVYTRDEVTGEAALRGTTLQSLGLTGGSATIRFVMKCYDP
VGKTPGSLGSSASAGQAAASAPLPLESGELSRGDLSRPEDADTSGPCCEH
TQEKQSTRAPAAAPFVPFSGGGQRLGGPPGPTRPLTSSSAKLPKSLSSPG
GPSKPKKSKSGQDPQQEQEQERERDPQQEQERERPVDREPVDREPVVCHP
DLEERLQAWPAELPDEFFELTVDDVRRRLAQLKSERKRLEEAPLVTKAFR
EAQIKEKLERYPKVALRVLFPDRYVLQGFFRPSETVGDLRDFVRSHLGNP
ELSFYLFITPPKTVLDDHTQTLFQANLFPAALVHLGAEEPAGVYLEPGLL
EHAISPSAADVLVARYMSRAAGSPSPLPAPDPAPKSEPAAEEGALVPPEP
IPGTAQPVKRSLGKVPKWLKLPASKR*

Figure 8D

mmL2:
MKKFFQEIKADIKFKSAGPGQKLTDSAGEKTTKGKSPQLALRQPRQGPTD
EAQMAAAAALARLEQKQPRARGPTSQDSIRNQVRKELQAEATSSNNPGAP
GTNSVPEPKEEISPHLAVPGVFFICPLTGVTLRRDQRDAHIKQAILSHFS
TDPVAASIMKIHTFNRDRDRVKLGVDTIAKYLDNIHLHPEEEKYQKIKLQ
NKVFQERINCLEGSHEFFEAIGFKKVTLPVPDQEGQEEFYVLGEDARAPQ
NLARHKQQLLDAEPVRATLDRQLRVFRPSALASHFELPSDFFSLTAEEVK
RDERLRTEAVERLSSLRTKAMREKEEQRDVRKYTYALVRVRLPDGCLLQG
TFYAREKLSALFRFVREALQNDWLPFELRASGGQKLEENEALALNECGLV
PSALLTFSWDASVLEDIRAAGAEPAKSVLRPELLAAIEQLS*

Figure 8E

hsL2:
MKKFFQEFKADIKFKSAGPGQKLKESVGEKAHKEKPNQPAPRPPRQGPTN
EAQMAAAAALARLEQKQSRAWGPTSQDTIRNQVRKELQAEATVSGSPEAP
GTNVVSEPREEGSAHLAVPGVYFTCPLTGATLRKDQRDACIKEAILLHFS
TDPVAASIMKIYTFNKDQDRVKLGVDTIAKYLDNIHLHPEEEKYRKIKLQ
NKVFQERINCLEGTHEFFEAIGFQKVLLPAQDQEDPEEFYVLSETTLAQP
QSLERHKEQLLAAEPVRAKLDRQRRVFQPSPLASQFELPGDFFNLTAEEI
KREQRLRSEAVERLSVLRTKAMREKEEQRGLRKYNYTLLRVRLPDGCLLQ
GTFYARERLGAVYGFVREALQSDWLPFELLASGGQKLSEDENLALNECGL
VPSALLTFSWDMAVLEDIKAAGAEPDSILKPELLSAIEKLL*

Figure 8F

hsL1 short splice variant:
MVPASRSREGPENMVRIALQLDDGSRLQDSFCSGQTLWELLSHFPQIREC
LQHPGGATPVCVYTRDEVTGEAALRGTTLQSLGLTGGSATIRFVMKCYDP

Figure 8G

```
1/1                                     31/11                                   61/21                                   91/31
TCA GCG GGT TTA AAC TCA ATG GTG ATG GTG ATG ATG ACC GGT ACG CGT AGA ATC GAG ACC GAG GAG AGG GTT AGG GAT AGG CTT ACC TTC GAA CCG CGG GCC CTC TAG ACT CGA GCG GCC

121/41                                  151/51                                  181/61                                  211/71
GCC ACT GTG CTG GAT ATC TGC AGA ATT GCC CTT AGC GCG TGA ATC AGA TCG GGG GTG GTG GCG GGG CTA GAC CTC TCT CTC CAG TGG AGA TTT GCC AAC CTG CCT AAC AAT GCC AAG CTG

241/81                                  271/91                                  301/101                                 331/111
GAG ATG GTG CCT GTG TCC CGT AGC CGC GAG GGG CCT GAG AAC ATA GTT CGC ATC GCG TTC CAG CTG GAT GAC GGC TCC AGG TTG CAG GAC GCT TTC TGT TCC CGA CAG ACT CTC TGG GAG
    M   V   P   V   S   R   S   R   E   G   P   E   N   I   V   R   I   A   F   Q   L   D   D   G   S   R   L   Q   D   A   F   C   S   R   Q   T   L   W   E
361/121                                 391/131                                 421/141                                 451/151
CTT CTC AGC CAT TTT GCA CAG ACC AGG GAG CGT CTG CAG CAG CTG GGT GAG AAG ACC CCT GTC TGC GTG TAC ATG AGG AAT GAG GTG ACT GGT AGA GCT GCC TTA CAG AAC ACA ACC CTT
L   L   S   H   F   A   Q   T   R   E   R   L   Q   Q   L   G   E   K   T   P   V   C   V   Y   M   R   N   E   V   T   G   R   A   A   L   Q   N   T   T   L
481/161                                 511/171                                 541/181                                 571/191
CAG TCA CTG GGC CTG ACA GGT GGT AGT GCC ACC ATC AGG TTT GTC ATA AAG CAG TGT GAC ACC GCC GGC AAA CAG GAG TCC ATA GCC GTC AGG AGC AAG GCC CCG GGA AGT CCG GTC TCC
Q   S   L   G   L   T   G   G   S   A   T   I   R   F   V   I   K   Q   C   D   T   A   G   K   Q   E   S   I   A   V   R   S   K   A   P   G   S   P   V   S
601/201                                 631/211                                 661/221                                 691/231
TCC TTG TCA GCC GAC CAG GCA TCC AGT AGC ACA TTG CTT CCT CTG AAC TCT GGG GAG TTC AGC AGG GGA GAC CTA AAC CAT GAG GGT GAT GCA AAC ACC TCA GGG ACC GGC CTT GAG GGT
S   L   S   A   D   Q   A   S   S   S   T   L   L   P   L   N   S   G   E   F   S   R   G   D   L   N   H   E   G   D   A   N   T   S   G   T   G   L   E   G
721/241                                 751/251                                 781/261                                 811/271
GGC CCC AAA CCA ACG GAT GCT CAA ACA AAG CAA AGC ACC AGT GAG CCT GCA TCG GCC CCC TTC GTT CCC TTC TCT GGG GGG GGC CAG CGG CTG GGG GGC CCG TCT GCG TCC TTG AGA CCT
G   P   K   P   T   D   A   Q   T   K   Q   S   T   S   E   P   A   S   A   P   F   V   P   F   S   G   G   G   Q   R   L   G   G   P   S   A   S   L   R   P
841/281                                 871/291                                 901/301                                 931/311
CTA ACA TCA CCT TCG GCC AAT TCA TCC AAG TCT TTC TCT GGC CCT GGG GGC CCT TCT AAG CCC AAG AAG CCA AAG CCT GGT GAG GAG CCC CAG CAG GAG CCT GAA CCG CCC GTT GAC CGA
L   T   S   P   S   A   N   S   S   K   S   F   S   G   P   G   G   P   S   K   P   K   K   P   K   P   G   E   E   P   Q   Q   E   P   E   P   P   V   D   R
961/321                                 991/331                                 1021/341                                1051/351
GAT CCT GTG GTG TAC CAC CCT GAC CTA GAG GAC TTG CTA CAG CCC TGG CCG GCA GAG GTG CCT GAC GAG TTC TTT GAG GTG ACT GTG GAT GAT GTG AGG AGA CGC TTG GCC CAG CTC AAG
D   P   V   V   Y   H   P   D   L   E   D   L   L   Q   P   W   P   A   E   V   P   D   E   F   F   E   V   T   V   D   D   V   R   R   R   L   A   Q   L   K
1081/361                                1111/371                                1141/381                                1171/391
AGT GAG CGG AAG CGT CTG GAA GAA GCC CCC CTG GTG ACC AAG GCT TTC AGG GAG GCT CAG ATG AAG GAG AAA CTG GAA CGT TAC CCA AAG GTG GCT CTG CGG GTC CTA TTC CCT GAC CGC
S   E   R   K   R   L   E   E   A   P   L   V   T   K   A   F   R   E   A   Q   M   K   E   K   L   E   R   Y   P   K   V   A   L   R   V   L   F   P   D   R
1201/401                                1231/411                                1261/421                                1291/431
TAC ATC CTG CAG GGC TTC TTC CGC CCC AGT GAG ACA GTG GGG GAT CTT CGA GAC TTT GTT AGG AGC CAC CTG GGG AAC CCC GAG CTC TCC TTT TAC CTA TTC ATC GCT CCA CCC AAA ATG
Y   I   L   Q   G   F   F   R   P   S   E   T   V   G   D   L   R   D   F   V   R   S   H   L   G   N   P   E   L   S   F   Y   L   F   I   A   P   P   K   M
1321/441                                1351/451                                1381/461                                1411/471
GTC CTG GAT GAC CAC ACG CTG ACT CTC TTT CAG GCA AAC CTC TTC CCT GCT GCG CTT GTG CAC TTT GGA GCT GAA GAA CCA ACA GGT CTC TAT CTG GAA CCT GGG CTG CTA GAA CAC ACC
V   L   D   D   H   T   L   T   L   F   Q   A   N   L   F   P   A   A   L   V   H   F   G   A   E   E   P   T   G   L   Y   L   E   P   G   L   L   E   H   T
1441/481                                1471/491                                1501/501                                1531/511
GTC TCC CCA TCT ACA GCT GAT GTG CTG GTG GCC AGG TGT ATG TCC CGG GCT TCC GGG TCC CCA CCT CTG CTG CCA GCC CCT GAC CCT GTA TCC CTG GAG TCT GAG CCA ATC GCT GAG GAT
V   S   P   S   T   A   D   V   L   V   A   R   C   M   S   R   A   S   G   S   P   P   L   L   P   A   P   D   P   V   S   L   E   S   E   P   I   A   E   D
1561/521                                1591/531                                1621/541                                1651/551
GGG GCA CTG GGG CCC CCA GAG CCC ATC CAA GGG ACA GCC CAG CCT GTG AAG AGA AGC CTG GGC AAA GTA CCC AAG TGG CTG AAG CTG CCA GCC AGC AAG AGG TGA GAG CTA CCA GCC TGG
G   A   L   G   P   P   E   P   I   Q   G   T   A   Q   P   V   K   R   S   L   G   K   V   P   K   W   L   K   L   P   A   S   K   R   *
1681/561                                1711/571                                1741/581                                1771/591
AGA TGC CTA TCA CCA GCC ACA GGA CCC TAC CCC CAC CCA CCC CGA GCA GGA ATA AAG AGT CGC GCT TCT CTC AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
```

Figure 9A

Docket/App No.: 0399.2025-002
Title: Expression Cloning Method
Inventors:Jonathan S. Bogan, Harvey F. Lodish

```
1/1                                     31/11                                   61/21                                   91/31
ACC CAC GCG TGC GCC CAC GCG TCC GCC CAC GCG TCC GGG CGA AGC CGG CTG CGG TCA CGT GAG CGG AAG ATG GCG GCC CCG GCG GGC GGC GGG GGC TCC GCG GTG TCG GTG CTG GCC CCA
                                                                                         M   A   A   P   A   G   G   G   G   S   A   V   S   V   L   A   P
121/41                                  151/51                                  181/61                                  211/71
AAC GGG CGG CGC CAC ACG GTG AAG GTG ACG CCG AGC ACC GTG CTG CTG CAG GTA CTA GAA GAC ACA TGC CGG CGG CAA GAC TTC AAC CCC AGT GAA TAC GAC CTG AAG TTT CAG AGG ACG
N   G   R   R   H   T   V   K   V   T   P   S   T   V   L   L   Q   V   L   E   D   T   C   R   R   Q   D   F   N   P   S   E   Y   D   L   K   F   Q   R   T
241/81                                  271/91                                  301/101                                 331/111
GTG CTA GAC CTC TCT CTC CAG TGG AGA TTT GCC AAC CTG CCT AAC AAT GCC AAG CTG GAG ATG GTG CCT GTG TCC CGT AGC CGC GAG GGG CCT GAG AAC ATA GTT CGC ATC GCG TTC CAG
V   L   D   L   S   L   Q   W   R   F   A   N   L   P   N   N   A   K   L   E   M   V   P   V   S   R   S   R   E   G   P   E   N   I   V   R   I   A   F   Q
361/121                                 391/131                                 421/141                                 451/151
CTG GAT GAC GGC TCC AGG TTG CAG GAC GCT TTC TGT TCC CGA CAG ACT CTC TGG GAG CTT CTC AGC CAT TTT GCA CAG ACC AGG GAG CGT CTG CAG CAG CTG GGT GAG AAG ACC CCT GTC
L   D   D   G   S   R   L   Q   D   A   F   C   S   R   Q   T   L   W   E   L   L   S   H   F   A   Q   T   R   E   R   L   Q   Q   L   G   E   K   T   P   V
481/161                                 511/171                                 541/181                                 571/191
TGC GTG TAC ATG AGG AAT GAG GTG ACT GGT AGA GCT GCC TTA CAG AAC ACA ACC CTT CAG TCA CTG GGC CTG ACA GGT GGT AGT GCC ACC ATC AGG TTT GTC ATA AAG CAG TGT GAC ACC
C   V   Y   M   R   N   E   V   T   G   R   A   A   L   Q   N   T   T   L   Q   S   L   G   L   T   G   G   S   A   T   I   R   F   V   I   K   Q   C   D   T
601/201                                 631/211                                 661/221                                 691/231
GCC GGC AAA CAG GAG TCC ATA GCC GTC AGG AGC AAG GCC CCG GGA AGT CCG GTC TCC TCC TTG TCA GCC GAC CAG GCA TCC AGT AGC ACA TTG CTT CCT CTG AAC TCT GGG GAG TTC AGC
A   G   K   Q   E   S   I   A   V   R   S   K   A   P   G   S   P   V   S   S   L   S   A   D   Q   A   S   S   S   T   L   L   P   L   N   S   G   E   F   S
721/241                                 751/251                                 781/261                                 811/271
AGG GGA GAC CTA AAC CAT GAG GGT GAT GCA AAC ACC TCA GGG ACC GGC CTT GAG GGT GGC CCC AAA CCA ACG GAT GCT CAA ACA AAG CAA AGC ACC AGT GAG CCT GCA TCG GCC CCC TTC
R   G   D   L   N   H   E   G   D   A   N   T   S   G   T   G   L   E   G   G   P   K   P   T   D   A   Q   T   K   Q   S   T   S   E   P   A   S   A   P   F
841/281                                 871/291                                 901/301                                 931/311
GTT CCC TTC TCT GGG GGG GGC CAG CGG CTG GGG GGC CCG TCT GCG TCC TTG AGA CCT CTA ACA TCA CCT TCG GCC AAT TCA TCC AAG TCT TTC TCT GGC CCT GGG GGC CCT TCT AAG CCC
V   P   F   S   G   G   G   Q   R   L   G   G   P   S   A   S   L   R   P   L   T   S   P   S   A   N   S   S   K   S   F   S   G   P   G   G   P   S   K   P
961/321                                 991/331                                 1021/341                                1051/351
AAG AAG CCA AAG CCT GGT GAG GAG CCC CAG CAG GAG CCT GAA CCG CCC GTT GAC CGA GAT CCT GTG GTG TAC CAC CCT GAC CTA GAG GAC TTG CTA CAG CCC TGG CCG GCA GAG GTG CCT
K   K   P   K   P   G   E   E   P   Q   Q   E   P   E   P   P   V   D   R   D   P   V   V   Y   H   P   D   L   E   D   L   L   Q   P   W   P   A   E   V   P
1081/361                                1111/371                                1141/381                                1171/391
GAC GAG TTC TTT GAG GTG ACT GTG GAT GAT GTG AGG AGA CGC TTG GCC CAG CTC AAG AGT GAG CGG AAG CGT CTG GAA GAA GCC CCC CTG GTG ACC AAG GCT TTC AGG GAG GCT CAG|ATG
D   E   F   F   E   V   T   V   D   D   V   R   R   R   L   A   Q   L   K   S   E   R   K   R   L   E   E   A   P   L   V   T   K   A   F   R   E   A   Q  |M
1201/401                                1231/411                                1261/421                                1291/431
AAG GAG AAA CTG GAA CGT TAC CCA AAG GTG GCT CTG CGG GTC CTA TTC CCT GAC CGC TAC ATC CTG CAG GGC TTC TTC CGC CCC AGT GAG ACA GTG GGG GAT CTT CGA GAC TTT GTT AGG
K   E   K   L   E   R   Y   P   K   V   A   L   R   V   L   F   P   D   R   Y   I   L   Q   G   F   F   R   P   S   E   T   V   G   D   L   R   D   F   V   R
1321/441                                1351/451                                1381/461                                1411/471
AGC CAC CTG GGG AAC CCC GAG CTC TCC TTT TAC CTA TTC ATC GCT CCA CCC AAA ATG GTC CTG GAT GAC CAC ACG CTG ACT CTC TTT CAG GCA AAC CTC TTC CCT GCT GCG CTT GTG CAC
S   H   L   G   N   P   E   L   S   F   Y   L   F   I   A   P   P   K   M   V   L   D   D   H   T   L   T   L   F   Q   A   N   L   F   P   A   A   L   V   H
1441/481                                1471/491                                1501/501                                1531/511
TTT GGA GCT GAA GAA CCA ACA GGT CTC TAT CTG GAA CCT GGG CTG CTA GAA CAC ACC GTC TCC CCA TCT ACA GCT GAT GTG CTG GTG GCC AGG TGT ATG TCC CGG GCT TCC GGG TCC CCA
F   G   A   E   E   P   T   G   L   Y   L   E   P   G   L   L   E   H   T   V   S   P   S   T   A   D   V   L   V   A   R   C   M   S   R   A   S   G   S   P
1561/521                                1591/531                                1621/541                                1651/551
CCT CTG CTG CCA GCC CCT GAC CCT GTA TCC CTG GAG TCT GAG CCA ATC GCT GAG GAT GGG GCA CTG GGC CCC CCA GAG CCC ATC CAA GGG ACA GCC CAG CCT GTG AAG AGA AGC CTG GGC
P   L   L   P   A   P   D   P   V   S   L   E   S   E   P   I   A   E   D   G   A   L   G   P   P   E   P   I   Q   G   T   A   Q   P   V   K   R   S   L   G
1681/561                                1711/571                                1741/581                                1771/591
AAA GTA CCC AAG TGG CTG AAG CTG CCA GCC AGC AAG AGG TGA GAG CTA CCA GCC TGG AGA TGC CTA TCA CCA GCC ACA GGA CCC TAC CCC CAC CCA CCC CGA GCA GGA ATA AAG AGT CGC
K   V   P   K   W   L   K   L   P   A   S   K   R   *
1801/601
GCT TCT CTC AAA AAA AAA AAA AAA AAA
```

Figure 9B

```
>gi|13129078|ref|NP_076988.1| ASPL protein [Homo sapiens]
 gi|12862970|gb|AAK08959.2|AF324219_1 (AF324219) ASPL [Homo sapiens]
          Length = 553

 Score =  720 bits (1858), Expect = 0.0
 Identities = 440/569 (77%), Positives = 464/569 (81%), Gaps = 35/569 (6%)

mm L1 long Query: 1   MAAPAGGGGSAVSVLAPNGRRHTVKVTPSTVLLQVLEDTCRRQDFNPSEYDLKFQRTVLD 60
                       MAAPAGGGGSAVSVLAPNGRRHTVKVTPSTVLLQVLEDTCRRQDFNP EYDLKFQR+VLD
hs L1 long Sbjct: 1   MAAPAGGGGSAVSVLAPNGRRHTVKVTPSTVLLQVLEDTCRRQDFNPCEYDLKFQRSVLD 60

           Query: 61  LSLQWRFANLPNNAKLEMVPVSRSREGPENIVRIAFQLDDGSRLQDAFCSRQTLWELLSH 120
                       LSLQWRFANLPNNAKLEMVP SRSREGPEN+VRIA QLDDGSRLQD+FCS QTLWELLSH
           Sbjct: 61  LSLQWRFANLPNNAKLEMVPASRSREGPENMVRIALQLDDGSRLQDSFCSGQTLWELLSH 120

           Query: 121 FAQTRERLQQLGEKTPVCVYMRNEVTGRAALQNTTLQSLGLTGGSATIRFVIKQCDTAGK 180
                       F Q RE LQ   G  TPVCVY R+EVTG AAL+ TTLQSLGLTGGSATIRFV+K  D  G
           Sbjct: 121 FPQIRECLQHPGGATPVCVYTRDEVTGEAALRGTTLQSLGLTGGSATIRFVMKCYDPVG- 179

           Query: 181 QESIAVRSKAPGSPVSSLSADQASSSTLLPLNSGEFSRGDLNHEGDANTSGTGLEGGPKP 240
                               K PGS  SS SA QA++S  LPL SGE SRGDL+   DA+TS      GP
           Sbjct: 180 --------KTPGSLGSSASAGQAAASAPLPLESGELSRGDLSRPEDADTS------GPCC 225

           Query: 241 TDAQTKQSTSEPASAPFVPFSGGGQRLGGPSASLRPLTSPSANSSKSFSGPGGPSKPKKP 300
                          Q KQST  PA+APFVPFSGGGQRLGGP    RPLTS SA   KS S PGGPSKPKK
           Sbjct: 226 EHTQEKQSTRAPAAAPFVPFSGGGQRLGGPPGPTRPLTSSSAKLPKSLSSPGGPSKPKKS 285

           Query: 301 KPGE------------EPQQEPE-------PPVDRDPVVYHPDLEDLLQPWPAEVPDEFF 341
                       K G+              +PQQE E         PVDR+PVV HPDLE+ LQ WPAE+PDEFF
           Sbjct: 286 KSGQDPQQEQEQERERDPQQEQERERPVDREPVDREPVVCHPDLEERLQAWPAELPDEFF 345

           Query: 342 EVTVDDVRRRLAQLKSERKRLEEAPLVTKAFREAQMKEKLERYPKVALRVLFPDRYILQG 401
                       E+TVDDVRRRLAQLKSERKRLEEAPLVTKAFREAQ+KEKLERYPKVALRVLFPDRY+LQG
           Sbjct: 346 ELTVDDVRRRLAQLKSERKRLEEAPLVTKAFREAQIKEKLERYPKVALRVLFPDRYVLQG 405

           Query: 402 FFRPSETVGDLRDFVRSHLGNPELSFYLFIAPPKMVLDDHTLTLFQANLFPAALVHFGAE 461
                       FFRPSETVGDLRDFVRSHLGNPELSFYLFI PPK VLDDHT TLFQANLFPAALVH GAE
           Sbjct: 406 FFRPSETVGDLRDFVRSHLGNPELSFYLFITPPKTVLDDHTQTLFQANLFPAALVHLGAE 465

           Query: 462 EPTGLYLEPGLLEHTVSPSTADVLVARCMSRASGSPPLLPAPDPVSLESEPIAEDGALGP 521
                       EP G+YLEPGLLEH +SPS ADVLVAR MSRA+GSP  LPAPDP   +SEP AE+GAL P
           Sbjct: 466 EPAGVYLEPGLLEHAISPSAADVLVARYMSRAAGSPSPLPAPDPAP-KSEPAAEEGALVP 524

           Query: 522 PEPIQGTAQPVKRSLGKVPKWLKLPASKR 550
                       PEPI GTAQPVKRSLGKVPKWLKLPASKR
           Sbjct: 525 PEPIPGTAQPVKRSLGKVPKWLKLPASKR 553
```

Figure 10

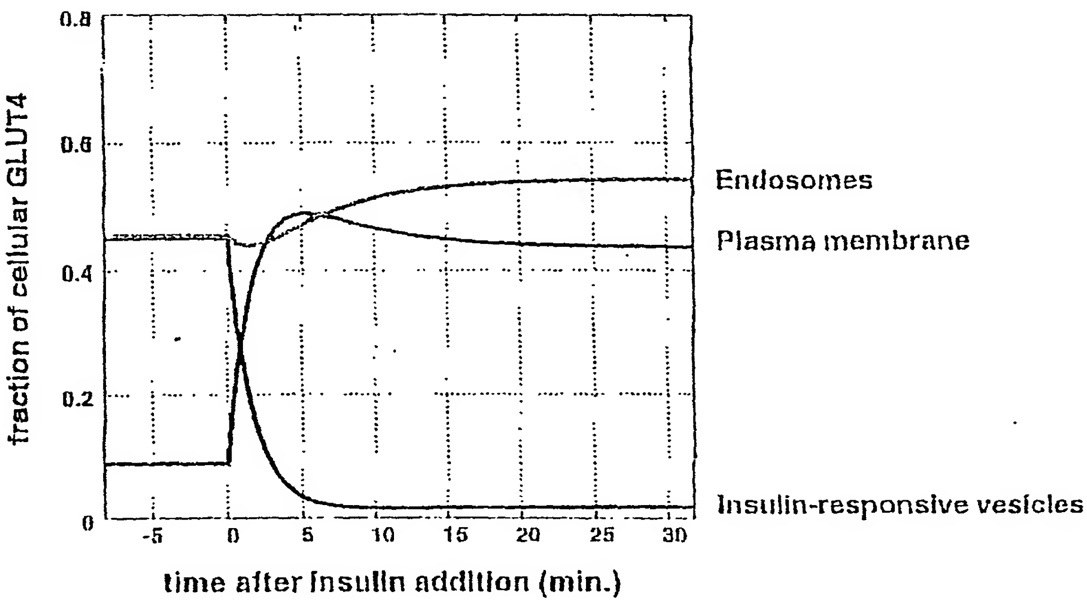

fraction of cellular GLUT4
0.8
0.6
0.4
0.2
0
-5
0
5
10
15
20
25
30
time after insulin addition (min.)
Endosomes
Plasma membrane
Insulin-responsive vesicles

Figure 11

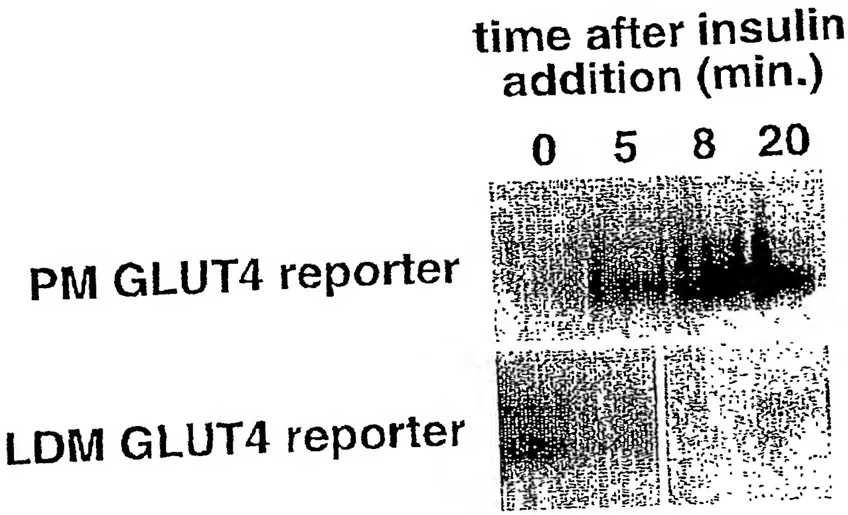


Figure 12

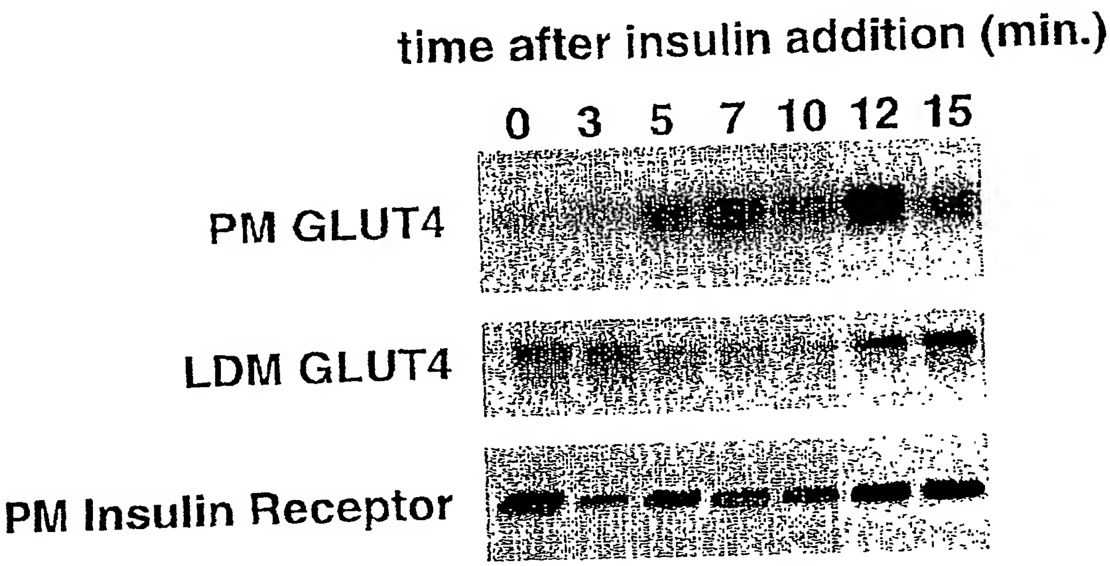


Figure 13

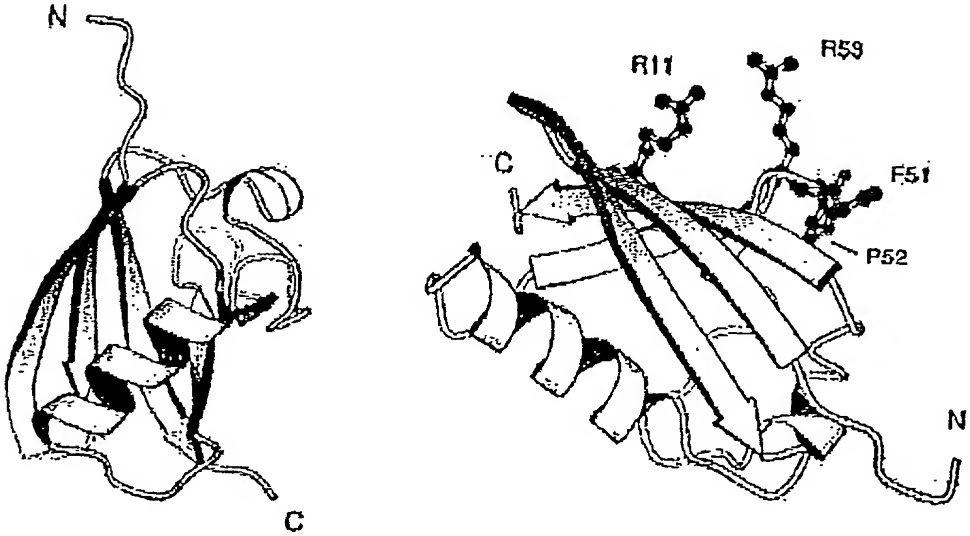


Figure 14

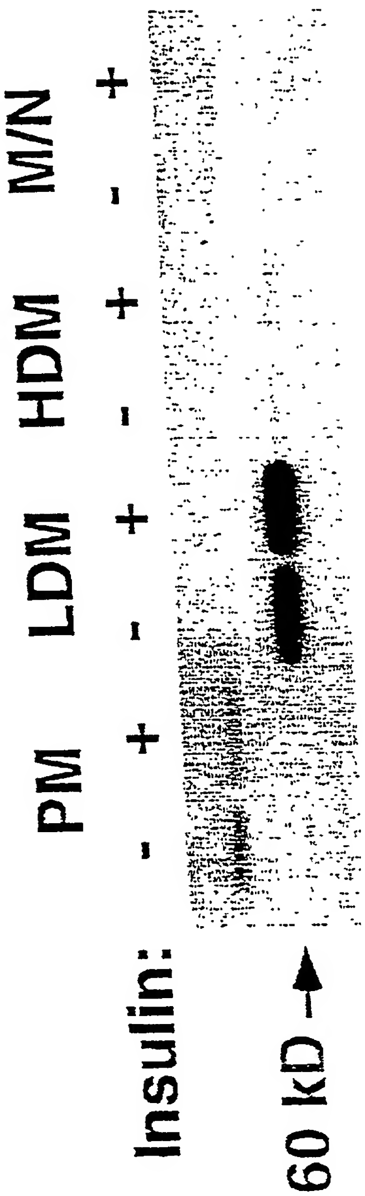


Figure 15

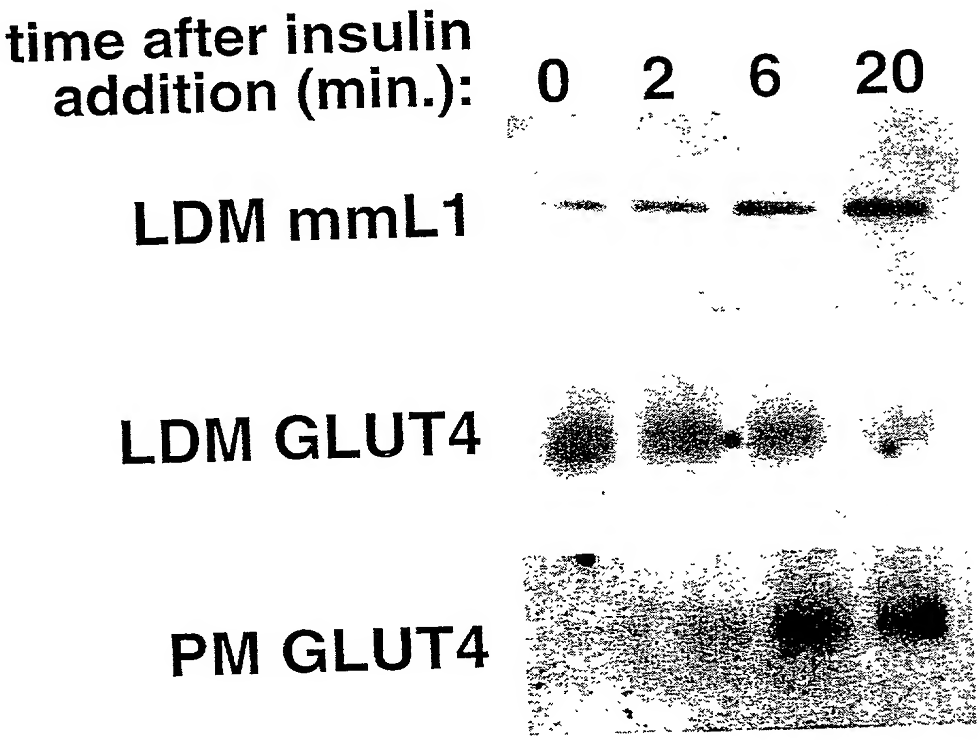

time after insulin addition (min.):
0 2 6 20
LDM mmL1
LDM GLUT4
PM GLUT4

Figure 16

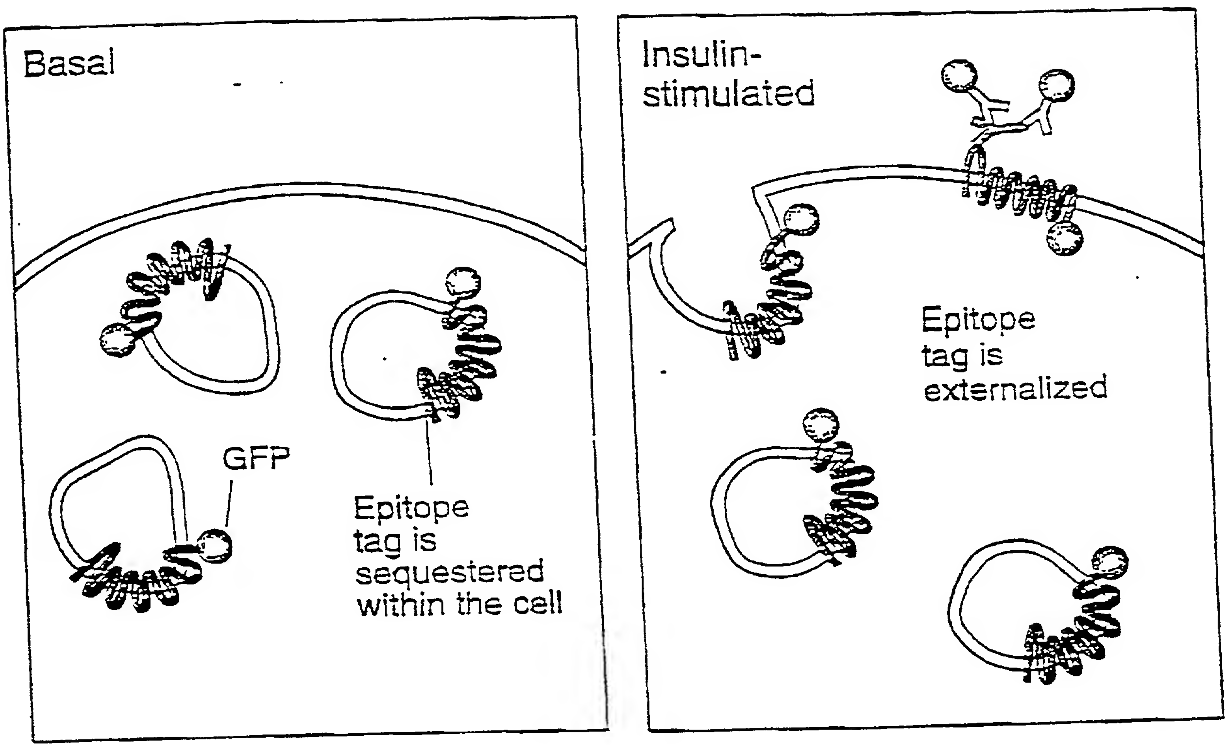

Basal
GFP
Epitope tag is sequestered within the cell
Insulin-stimulated
Epitope tag is externalized

Figure 17A

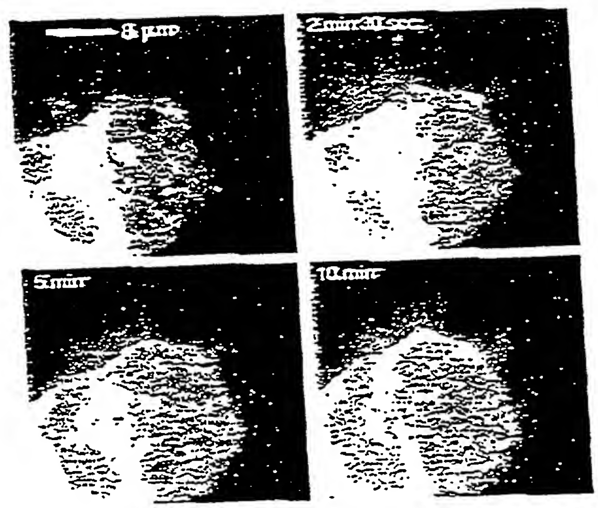


Figure 17B

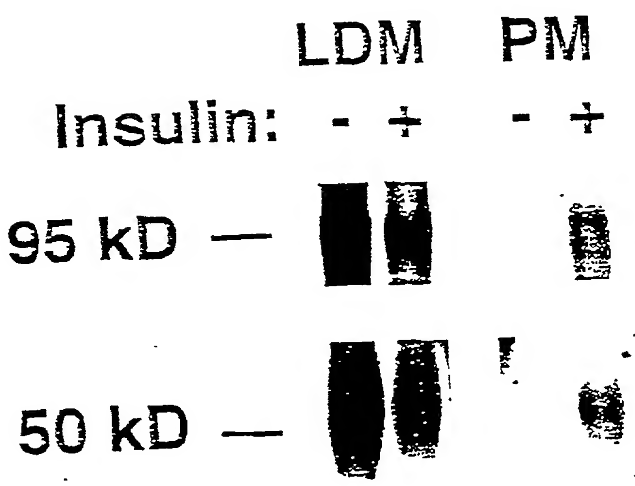


Figure 17C

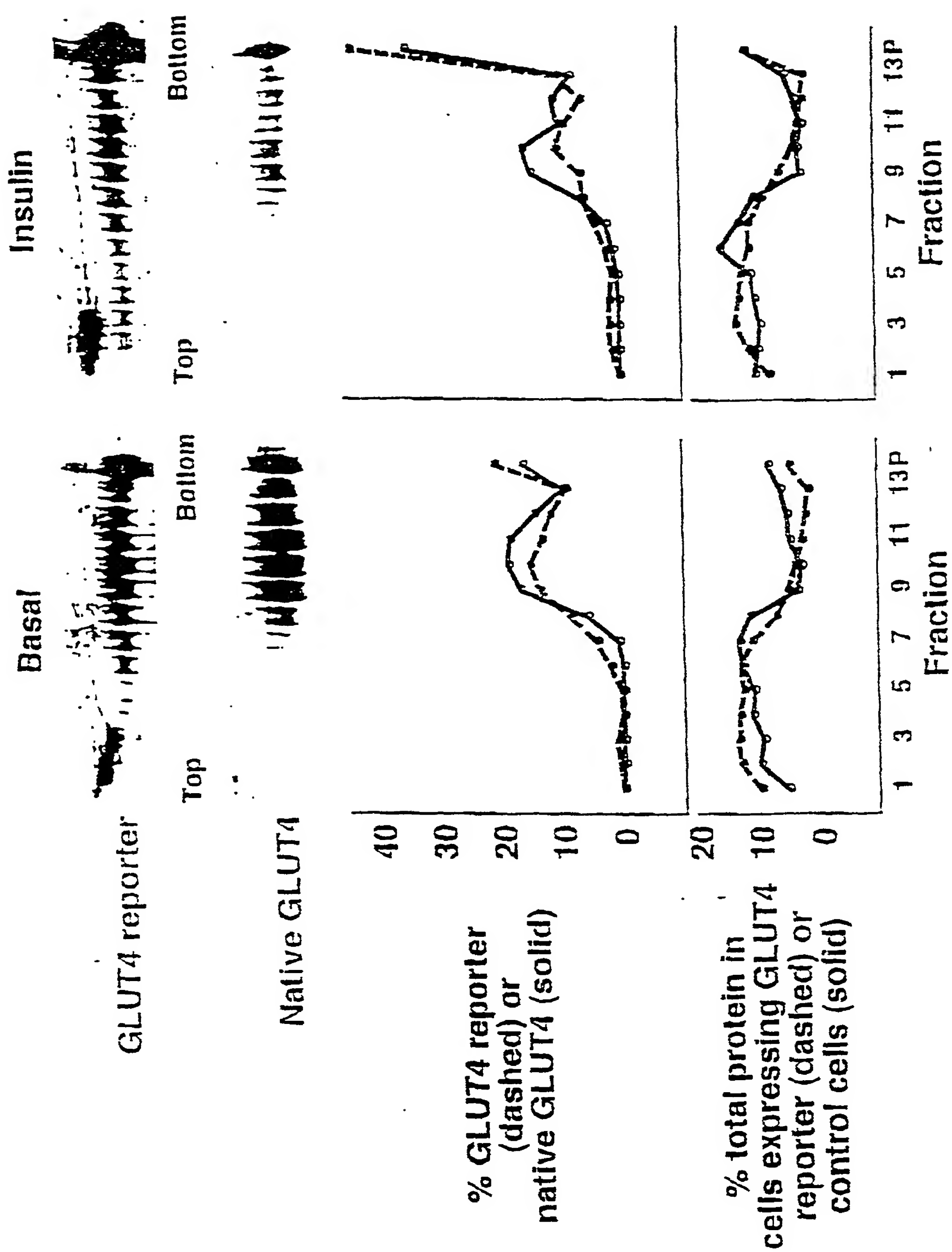

Basal
Insulin
GLUT4 reporter
Native GLUT4
Top
Bottom
Top
Bottom
% GLUT4 reporter (dashed) or native GLUT4 (solid)
40
30
20
10
0
% total protein in cells expressing GLUT4 reporter (dashed) or control cells (solid)
20
10
0
1 3 5 7 9 11 13P
Fraction
1 3 5 7 9 11 13P
Fraction

Figure 17D

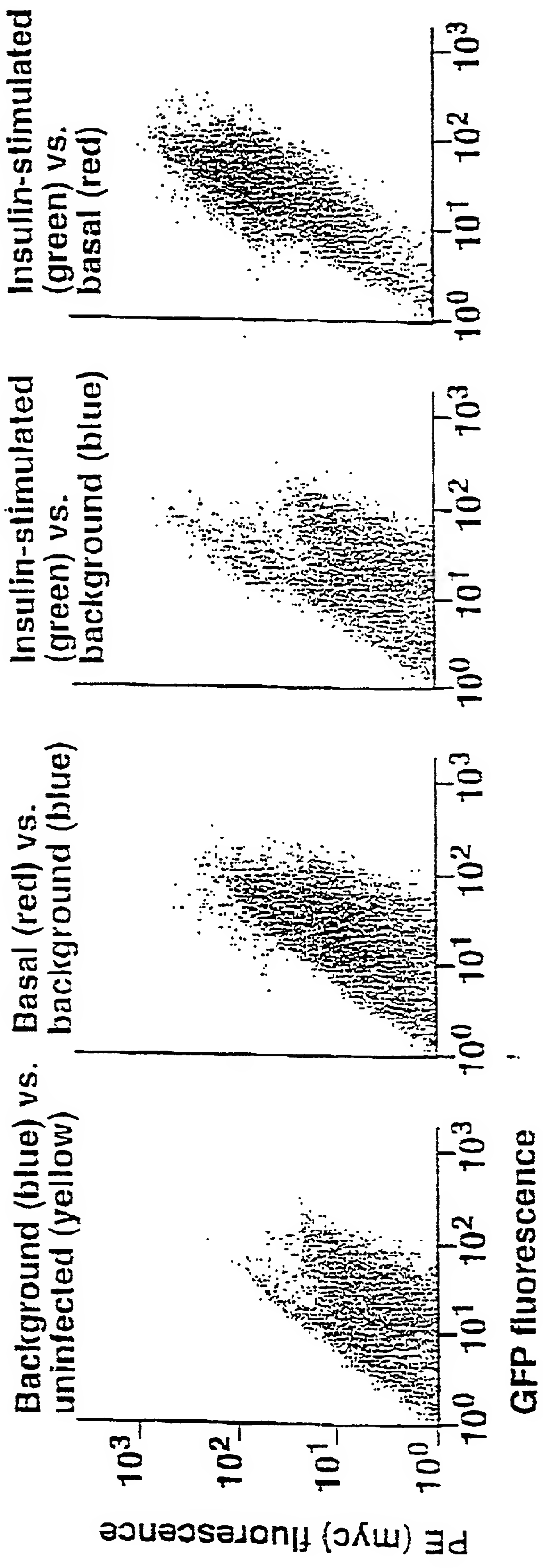

Background (blue) vs. uninfected (yellow)
Basal (red) vs. background (blue)
Insulin-stimulated (green) vs. background (blue)
Insulin-stimulated (green) vs. basal (red)
PE (myc) fluorescence
GFP fluorescence
10^0
10^1
10^2
10^3

Figure 17E

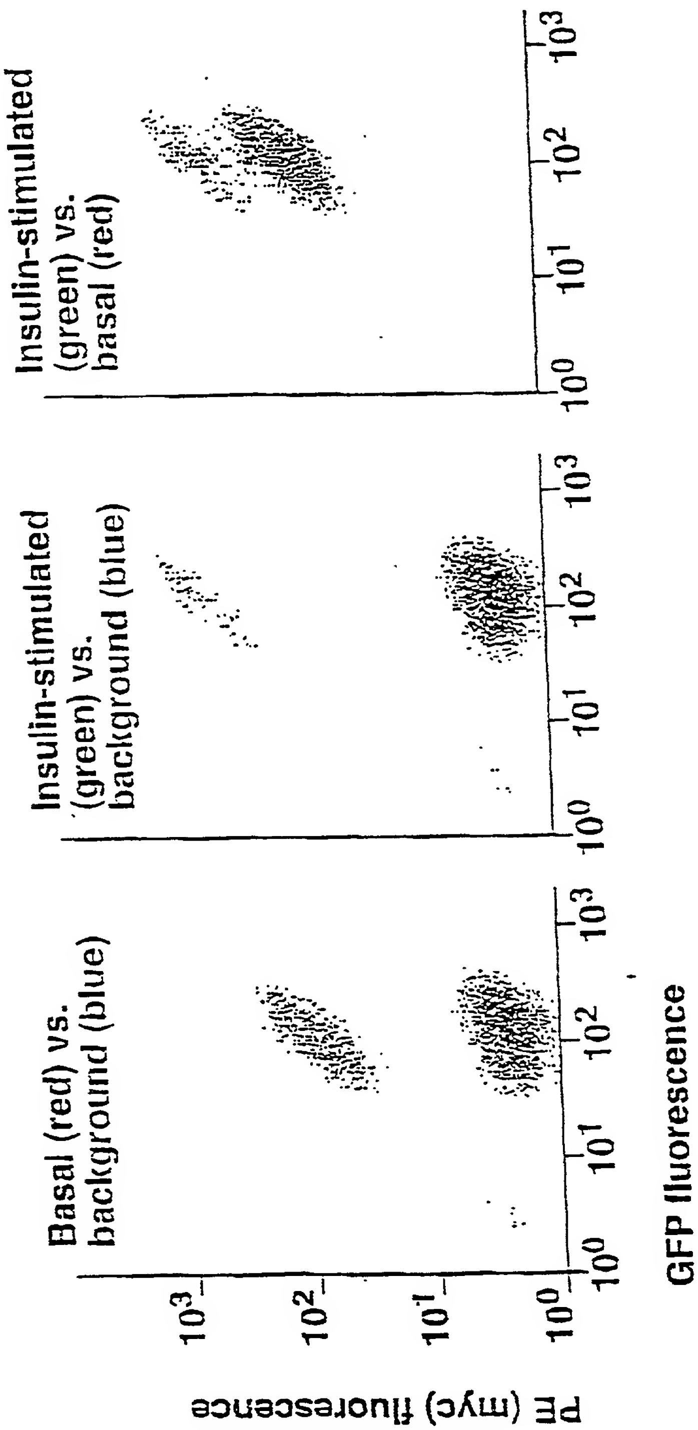

Basal (red) vs. background (blue)
Insulin-stimulated (green) vs. background (blue)
Insulin-stimulated (green) vs. basal (red)
PE (myc) fluorescence
GFP fluorescence
10^0
10^1
10^2
10^3

Figure 17F

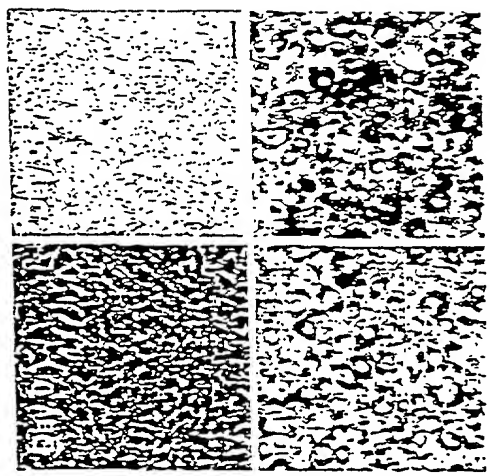


Figure 18B

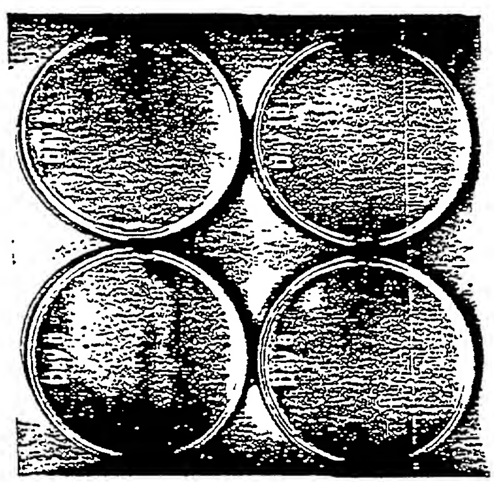


Figure 18A

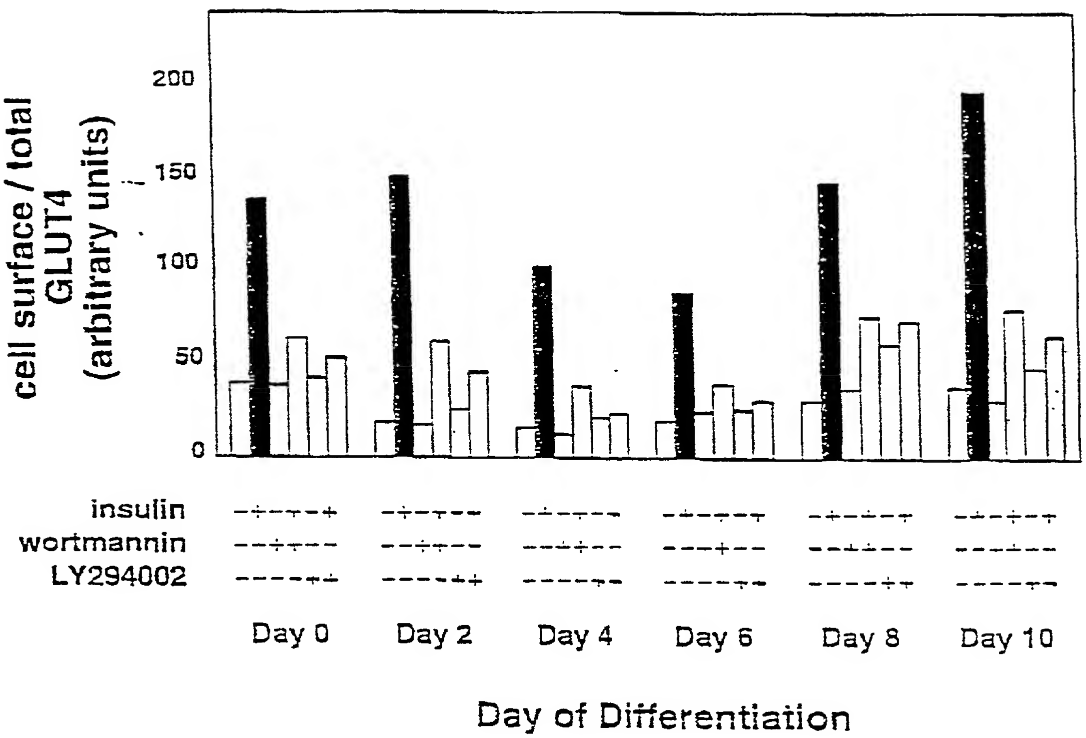

cell surface / total GLUT4 (arbitrary units)
200
150
100
50
0
insulin
wortmannin
LY294002
Day 0
Day 2
Day 4
Day 6
Day 8
Day 10
Day of Differentiation

Figure 18C

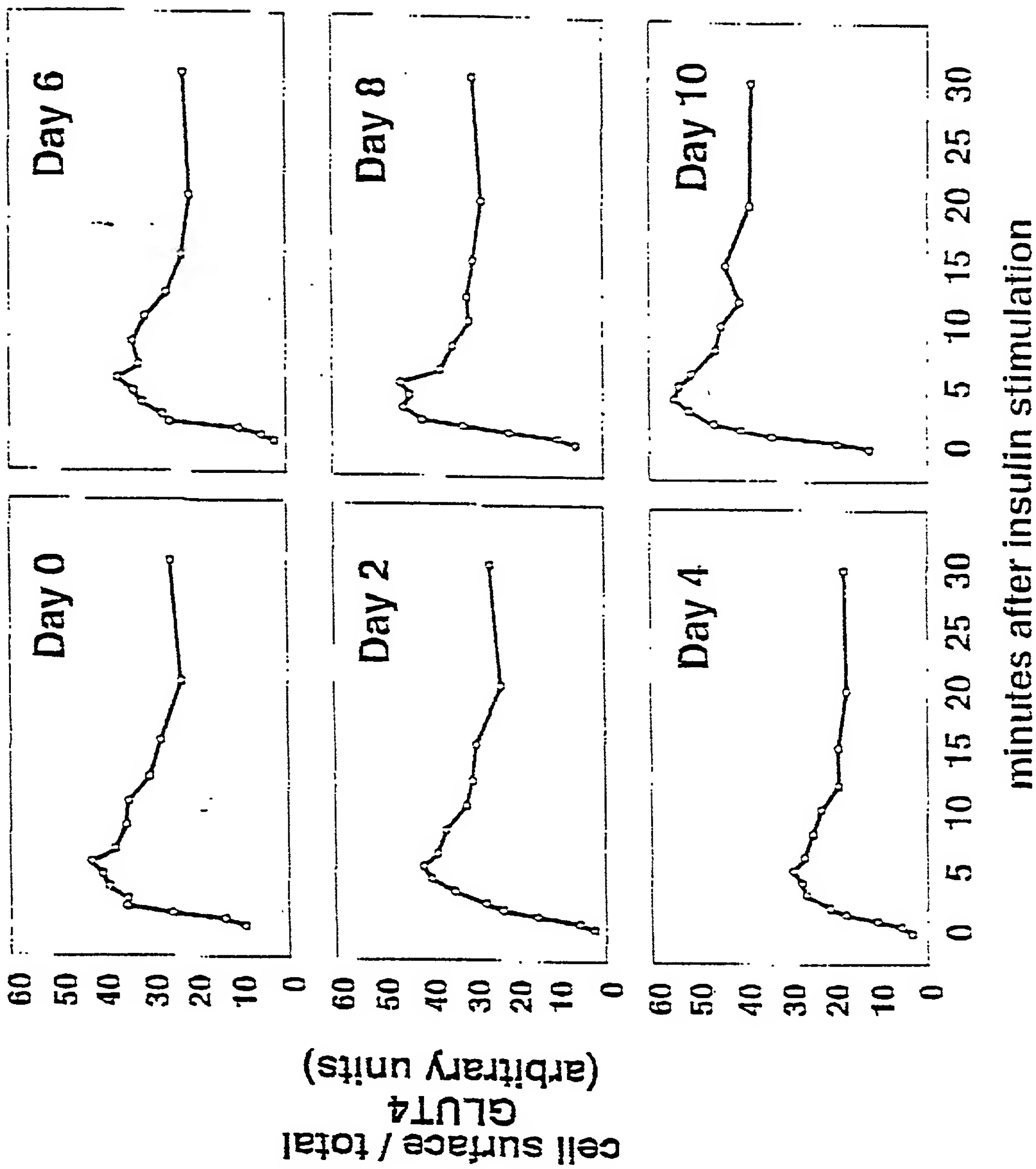

Day 0
Day 2
Day 4
Day 6
Day 8
Day 10
cell surface / total GLUT4 (arbitrary units)
0 10 20 30 40 50 60
minutes after insulin stimulation
0 5 10 15 20 25 30

Figure 19A

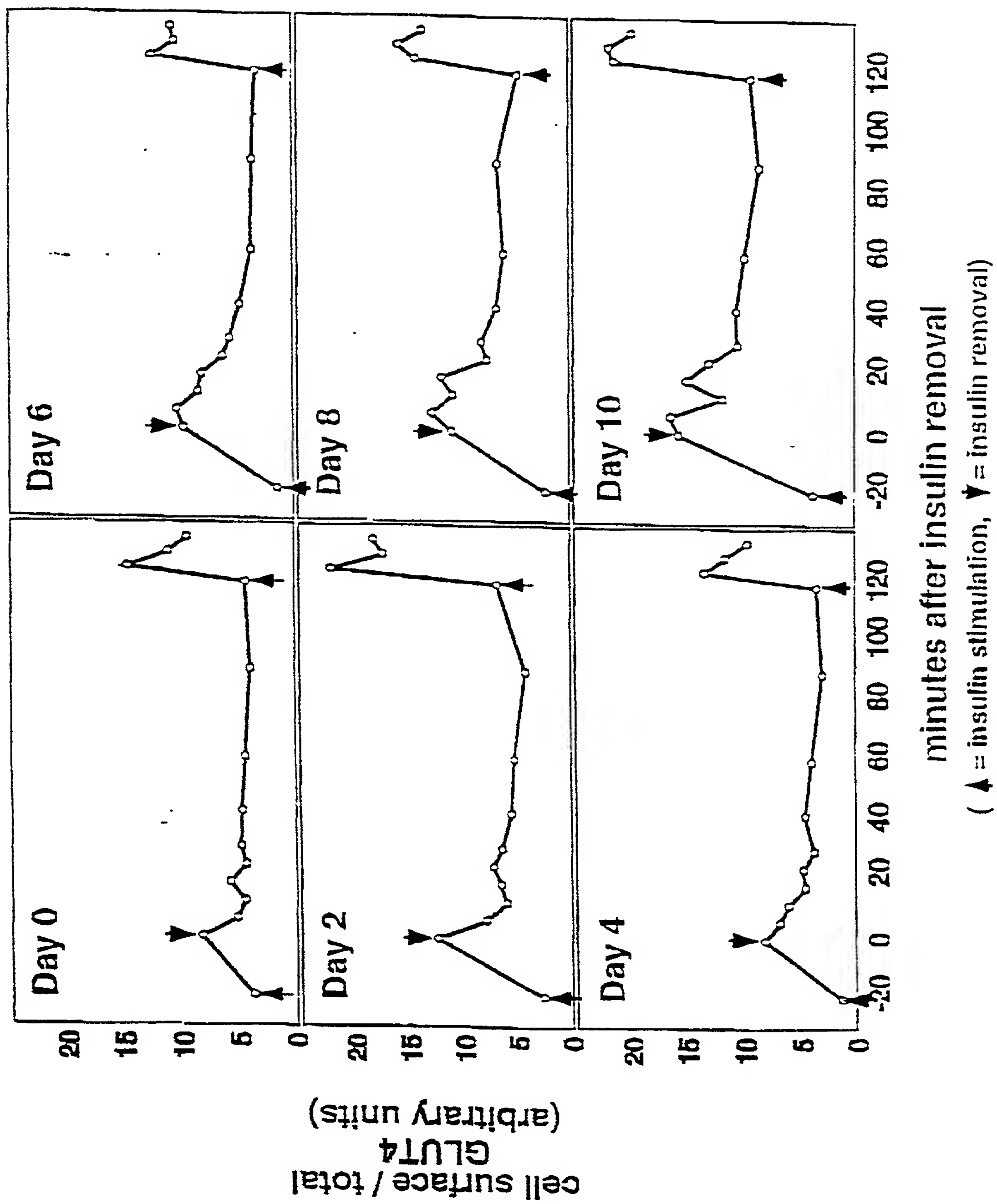

Day 0
Day 2
Day 4
Day 6
Day 8
Day 10
cell surface / total
GLUT4
(arbitrary units)
minutes after insulin removal
(▲ = insulin stimulation, ▼ = insulin removal)

Figure 19B

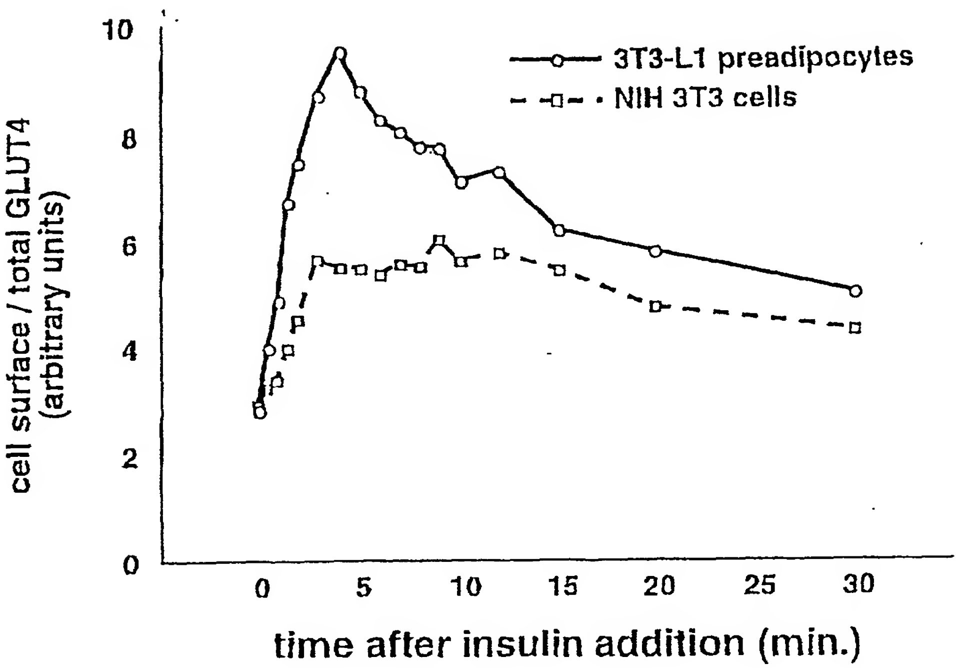

3T3-L1 preadipocytes
NIH 3T3 cells
cell surface / total GLUT4
(arbitrary units)
0
2
4
6
8
10
0
5
10
15
20
25
30
time after insulin addition (min.)

Figure 19C

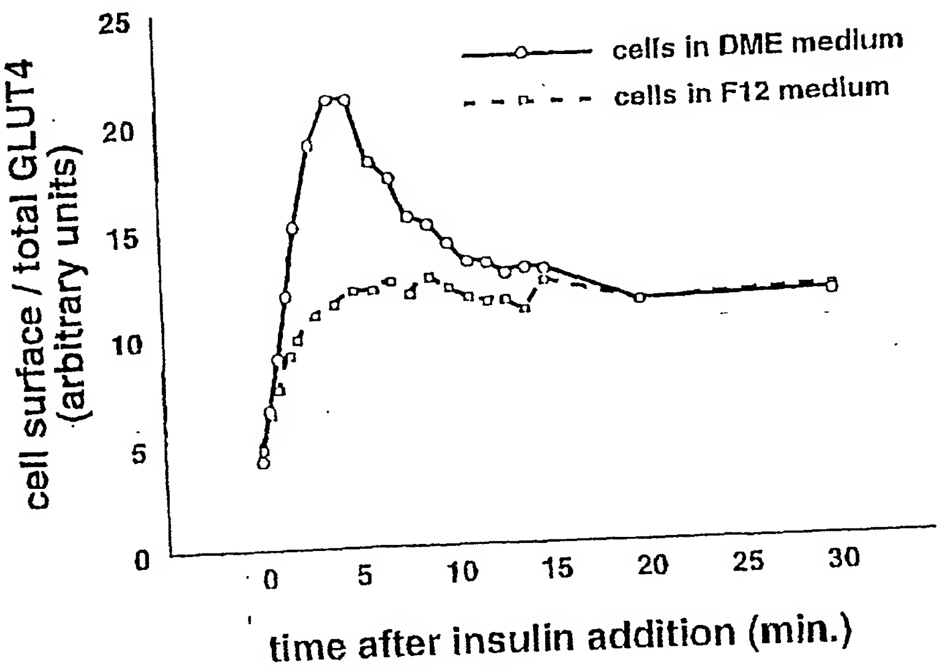

cells in DME medium
cells in F12 medium
cell surface / total GLUT4 (arbitrary units)
25
20
15
10
5
0
0
5
10
15
20
25
30
time after insulin addition (min.)

Figure 20

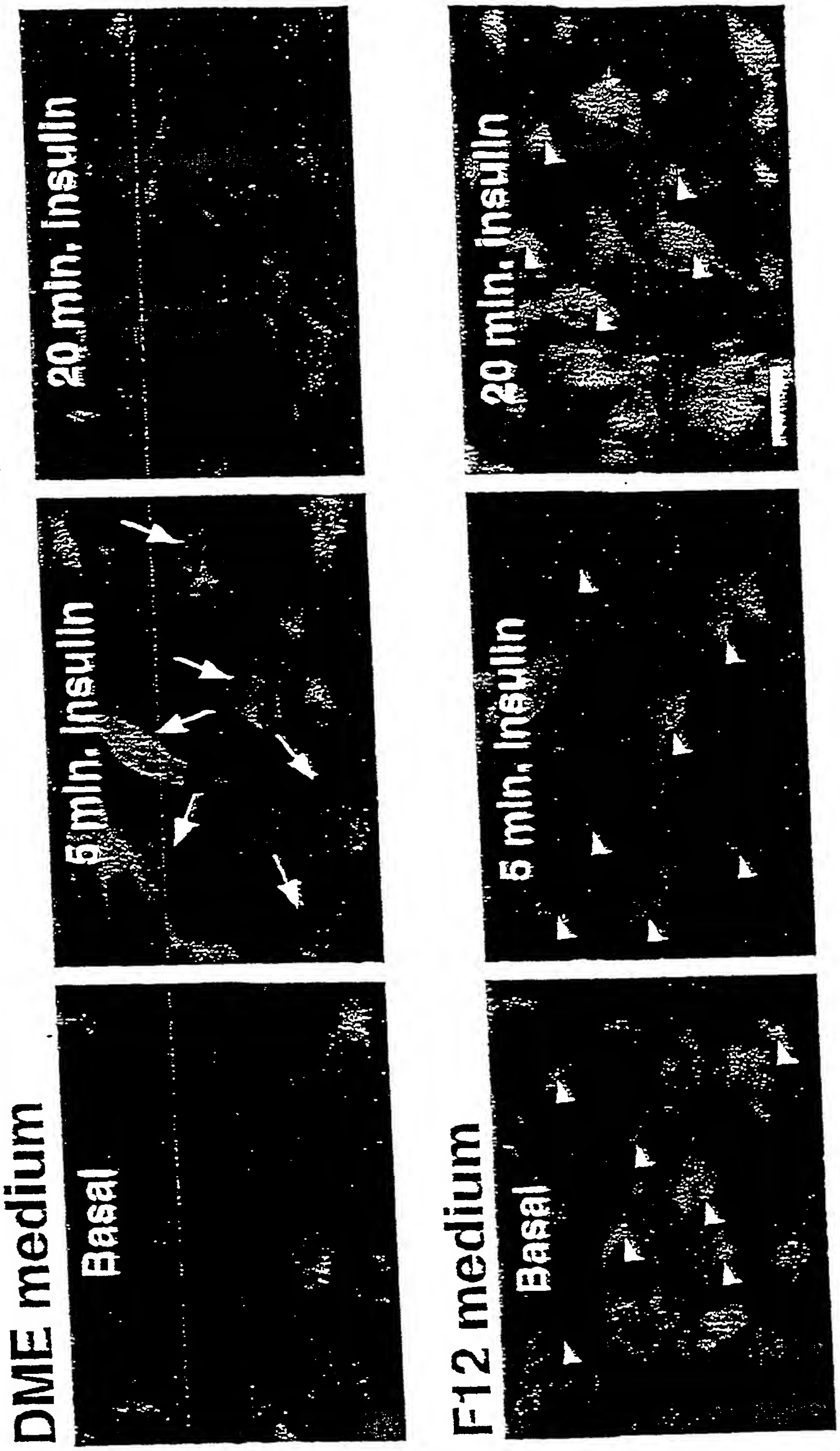

DME medium
Basal
5 min. Insulin
20 min. Insulin
F12 medium
Basal
5 min. Insulin
20 min. Insulin

Figure 21

Figure 22A

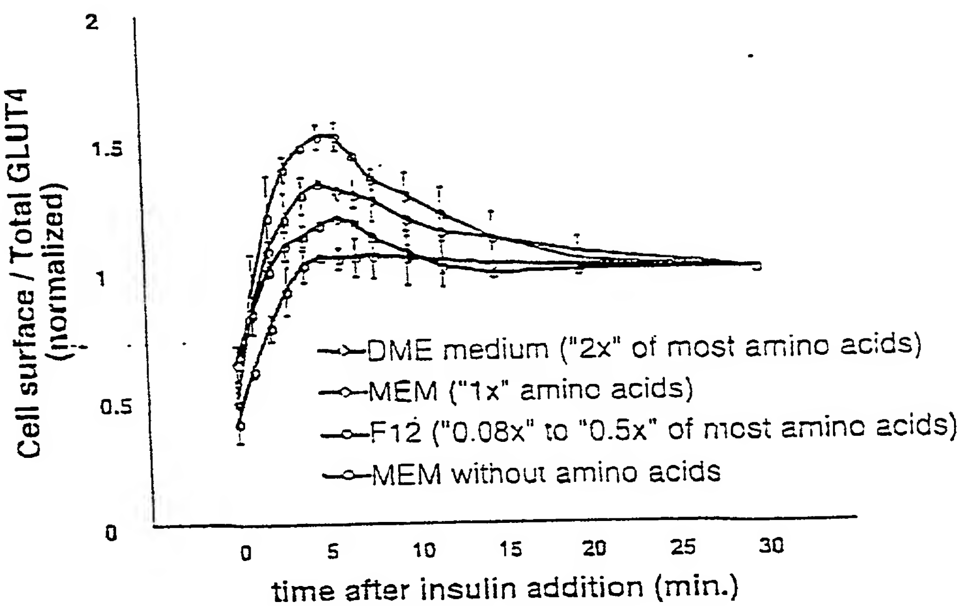
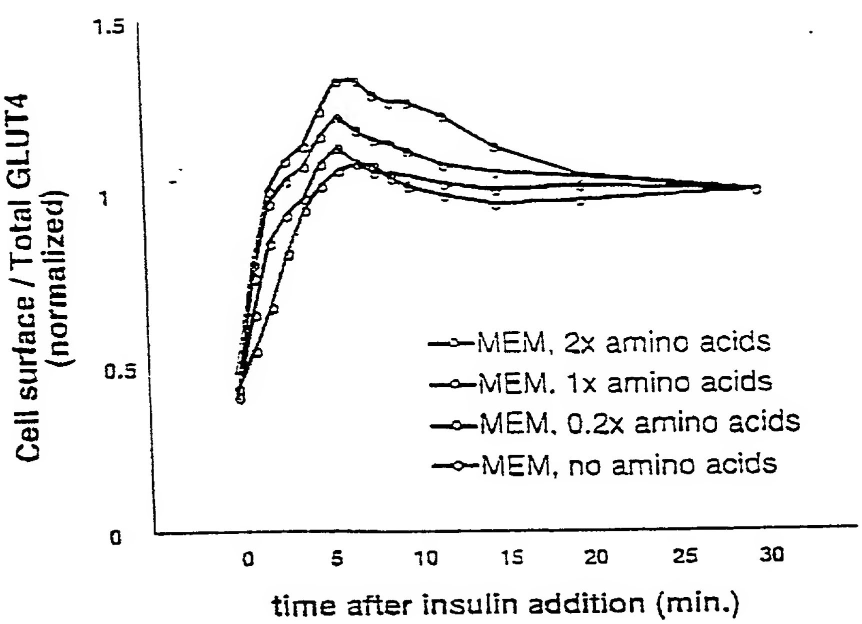


Figure 22B



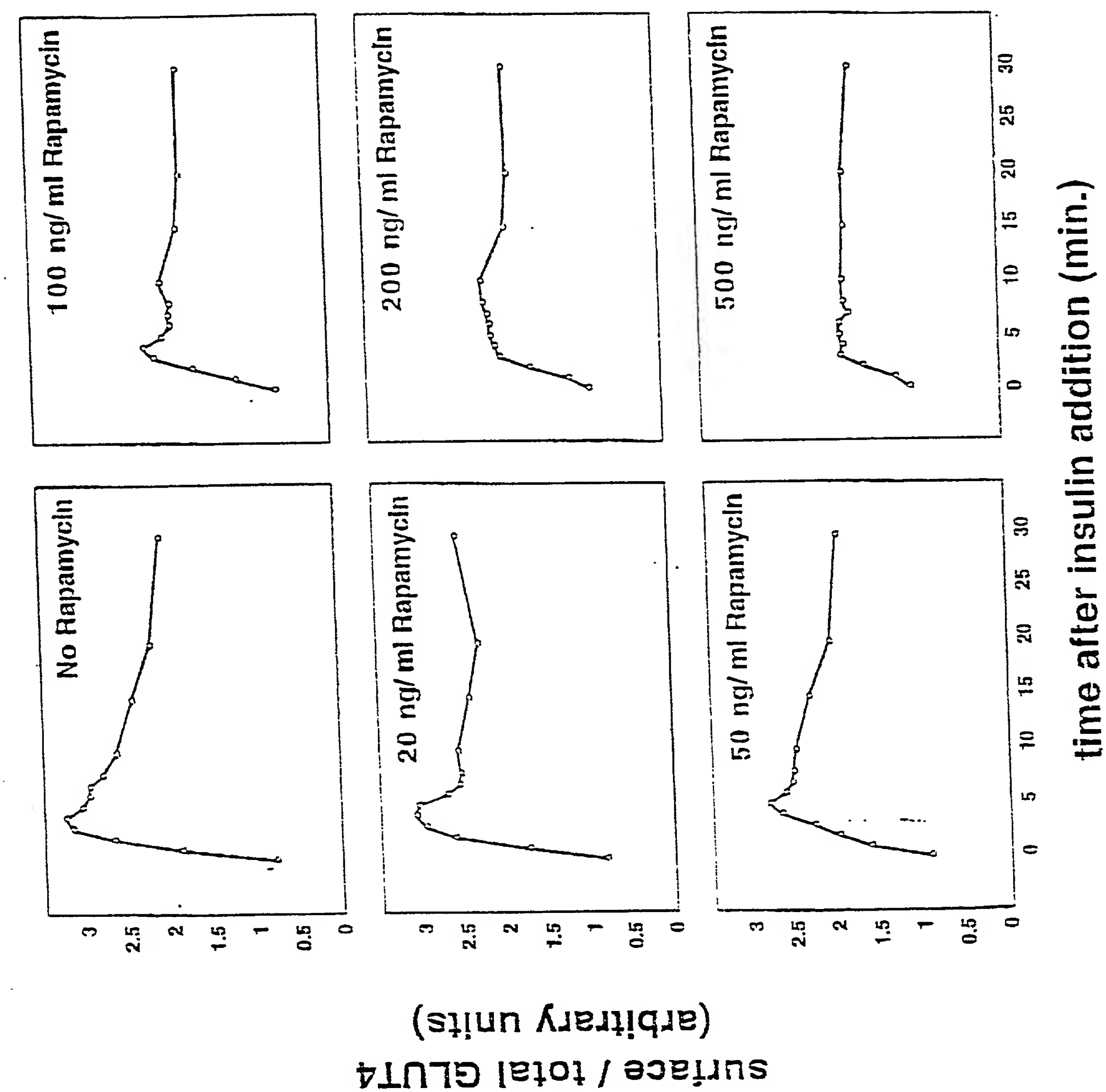

No Rapamycin
20 ng/ ml Rapamycin
50 ng/ ml Rapamycin
100 ng/ ml Rapamycin
200 ng/ ml Rapamycin
500 ng/ ml Rapamycin
surface / total GLUT4
(arbitrary units)
time after insulin addition (min.)
0
0.5
1
1.5
2
2.5
3
5
10
15
20
25
30

Figure 23

Figure 24

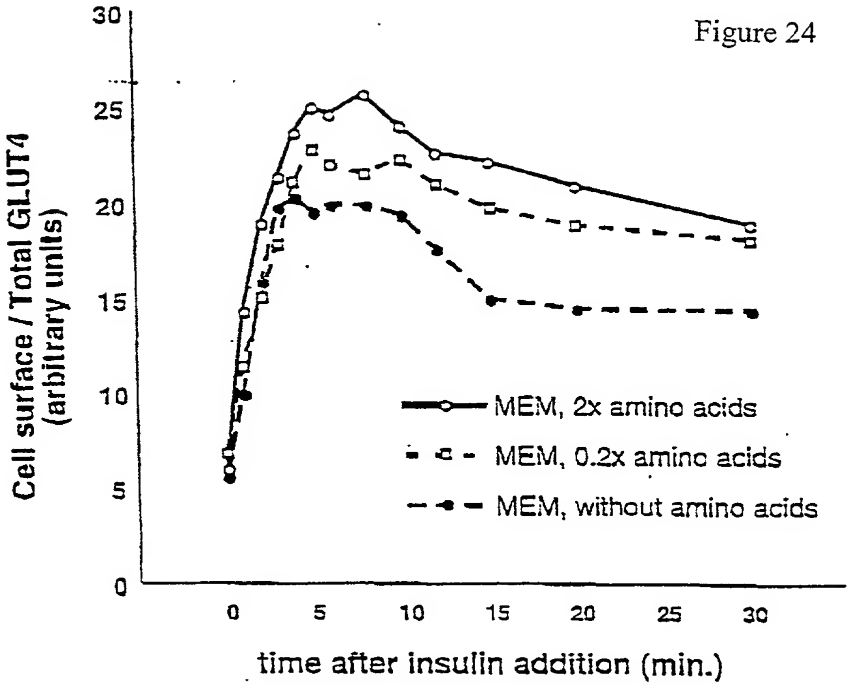
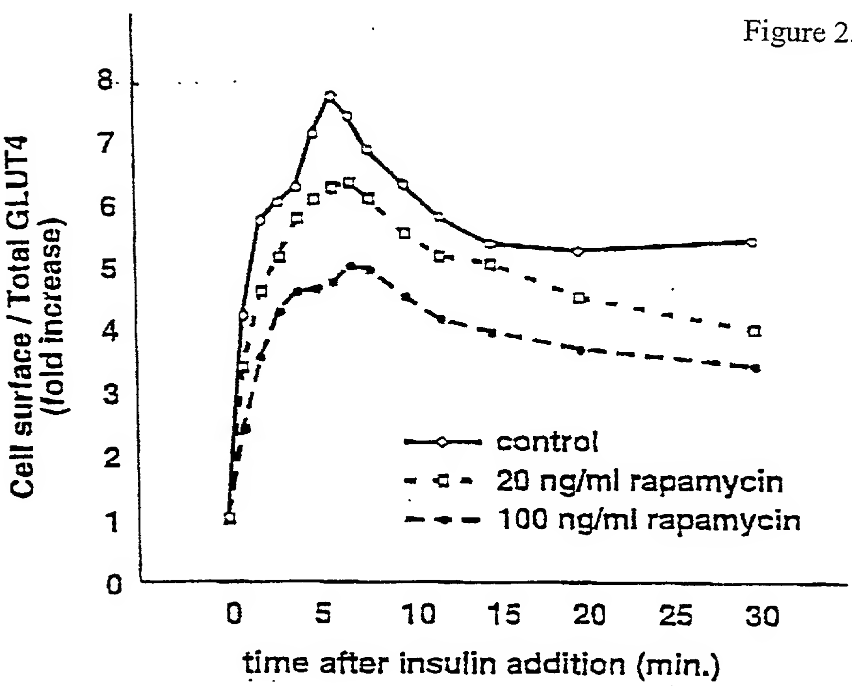


Figure 25



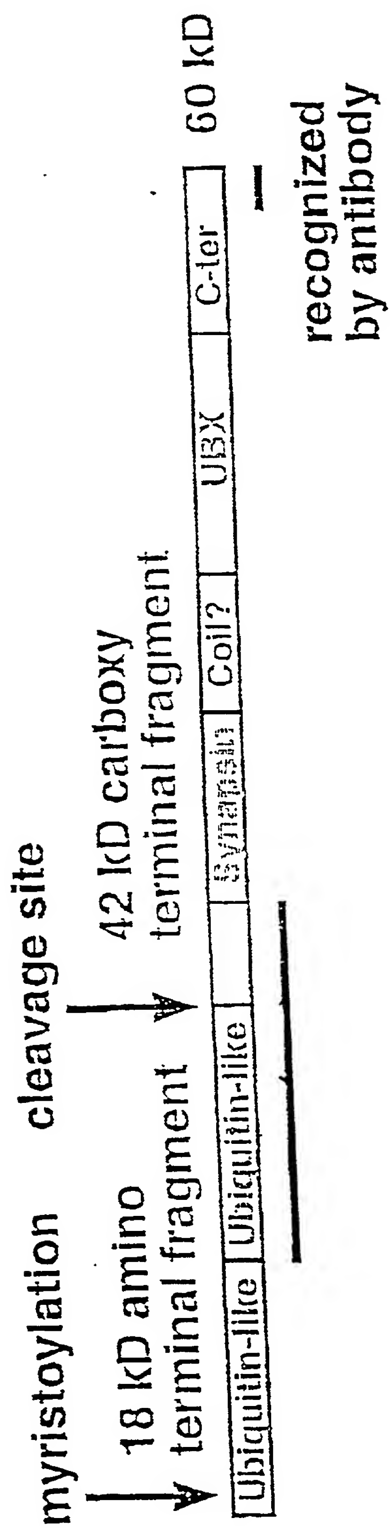

myristoylation
18 kD amino
terminal fragment
cleavage site
42 kD carboxy
terminal fragment
Ubiquitin-like
Ubiquitin-like
Coil?
UBX
C-ter
60 kD
recognized
by antibody

Figure 26

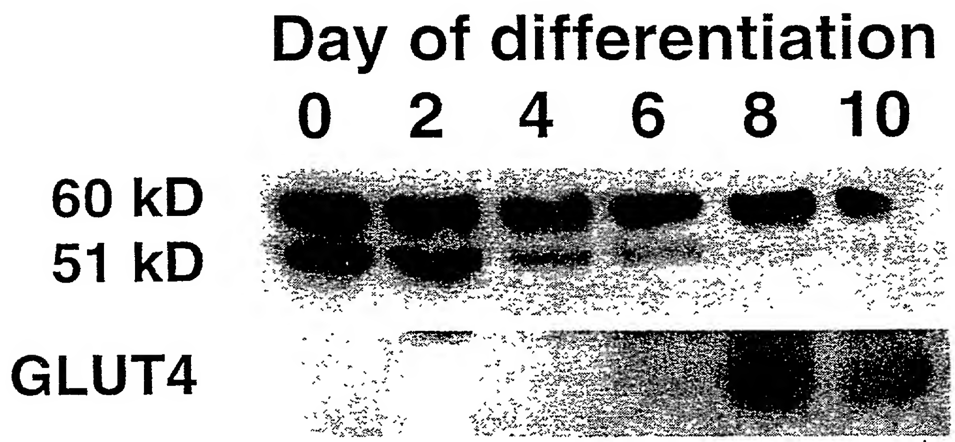


Figure 27

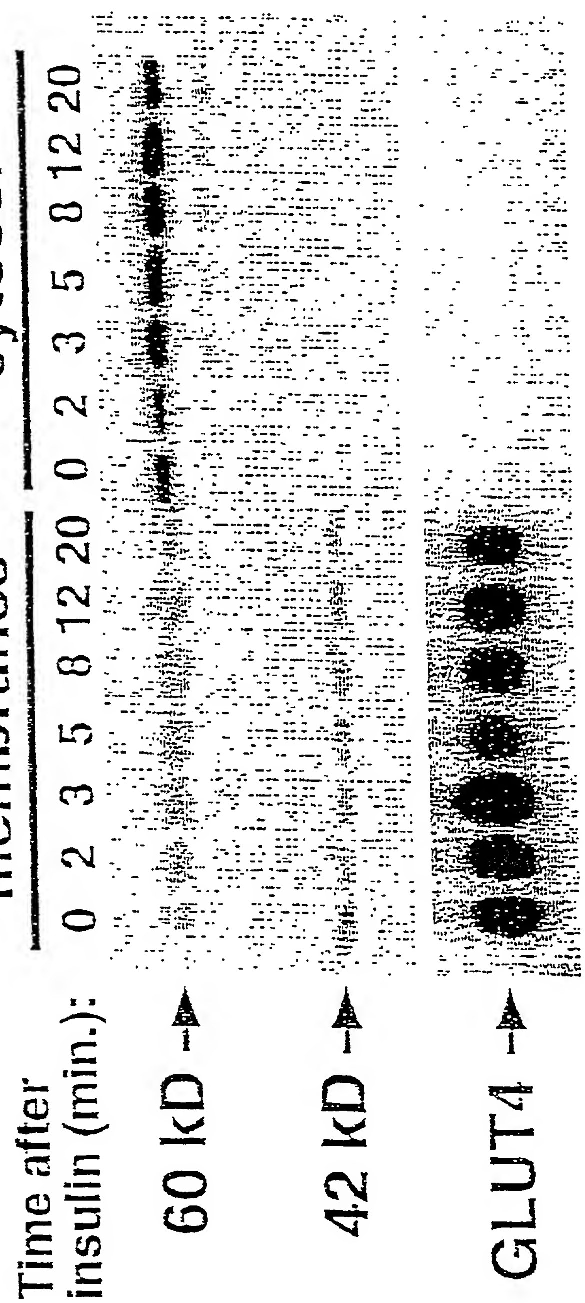

Time after insulin (min.):
membranes
cytosol
0 2 3 5 8 12 20
0 2 3 5 8 12 20
60 kD
42 kD
GLUT4

Figure 28

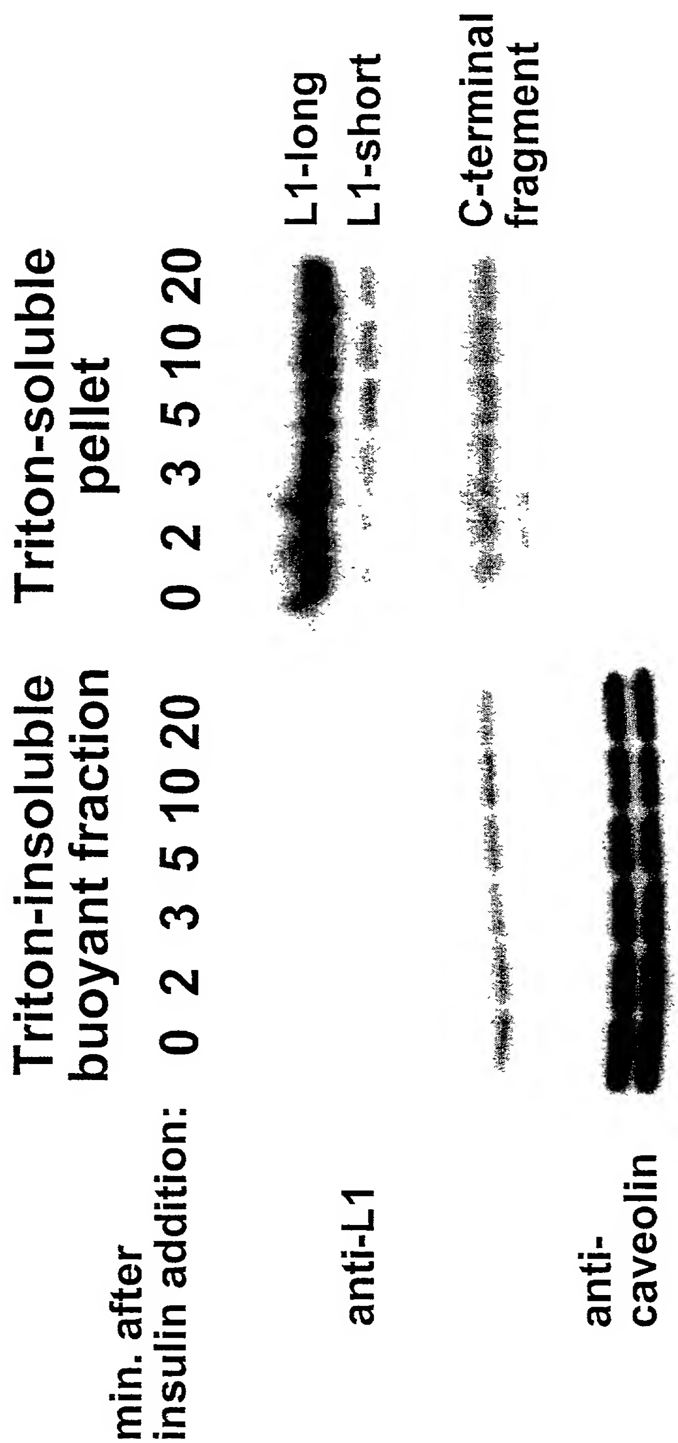

min. after insulin addition:
Triton-insoluble buoyant fraction
0 2 3 5 10 20
Triton-soluble pellet
0 2 3 5 10 20
anti-L1
anti-caveolin
L1-long
L1-short
C-terminal fragment

Figure 29

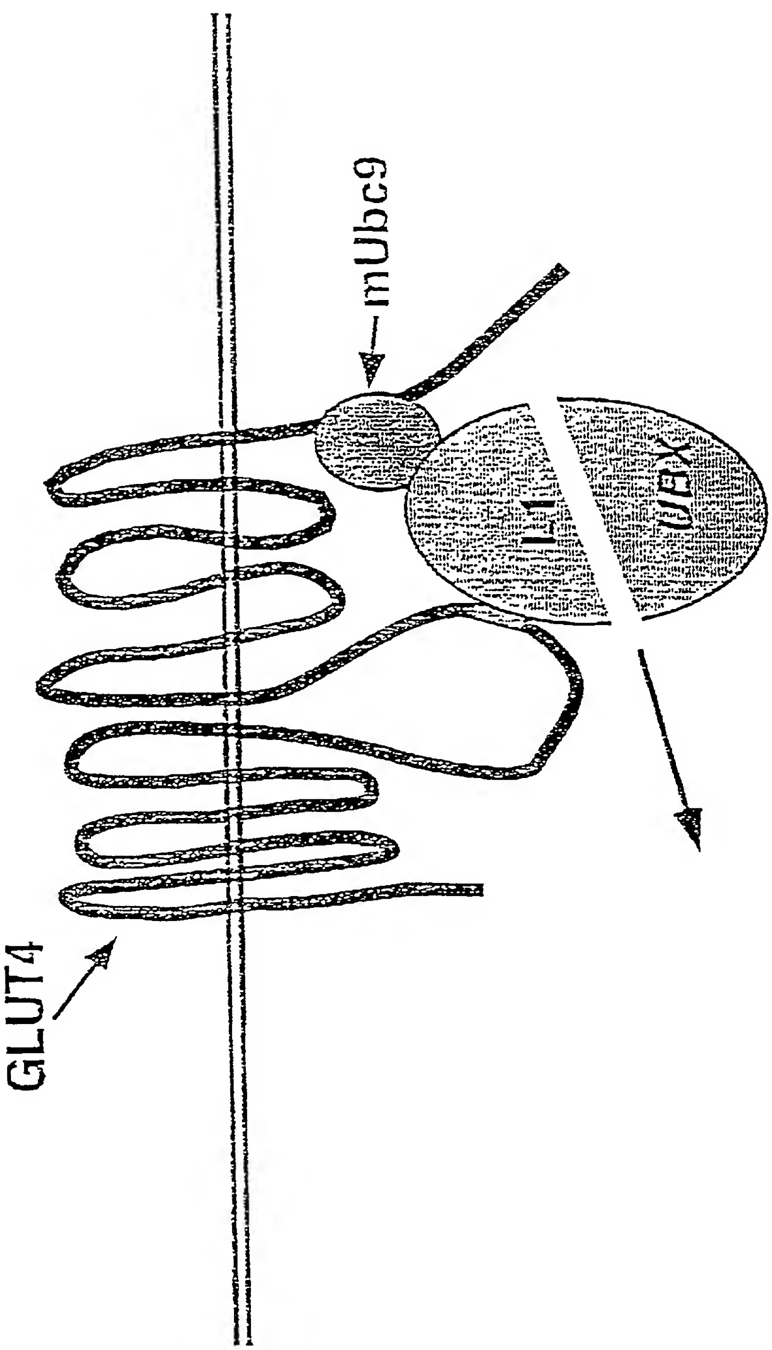


Figure 30

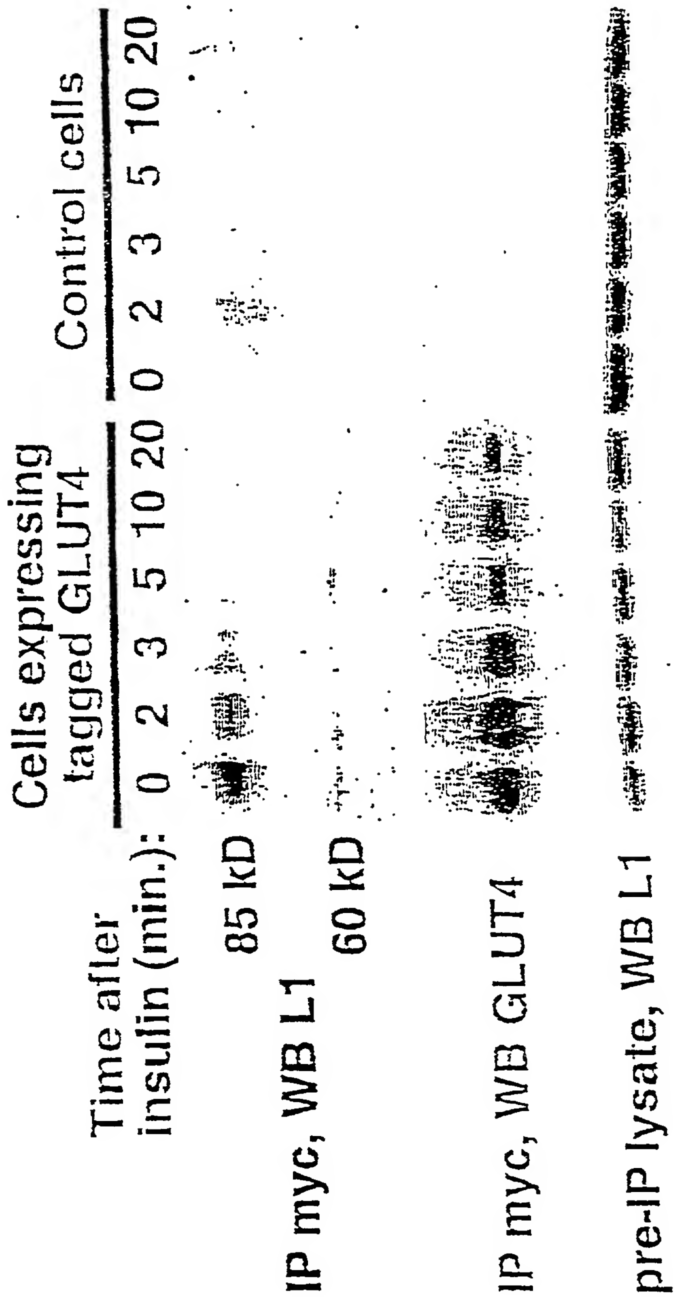

Time after insulin (min.):
Cells expressing tagged GLUT4
Control cells
0 2 3 5 10 20
0 2 3 5 10 20
85 kD
60 kD
IP myc, WB L1
IP myc, WB GLUT4
pre-IP lysate, WB L1

Figure 31

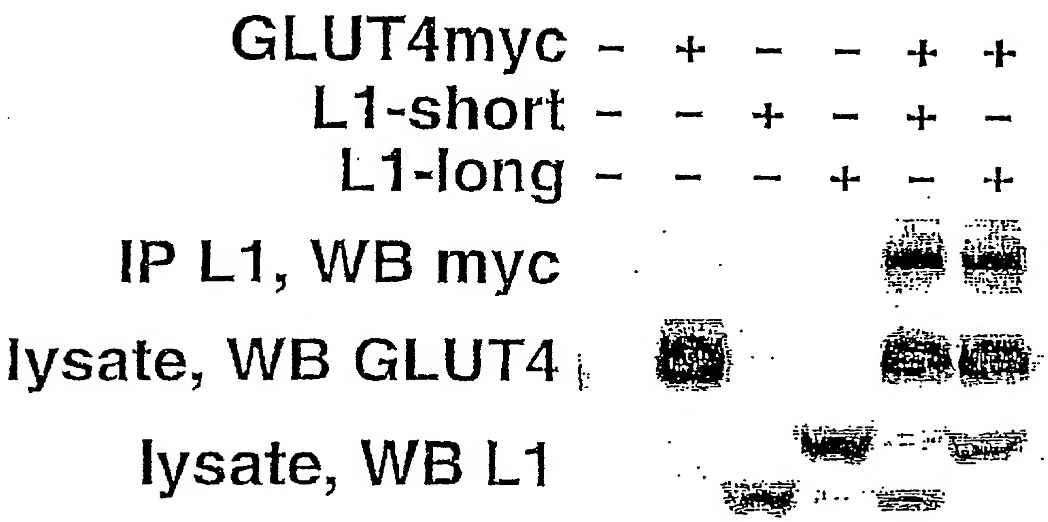

GLUT4myc – + – – + +
L1-short – – + – + –
L1-long – – – + – +
IP L1, WB myc
lysate, WB GLUT4
lysate, WB L1

Figure 32

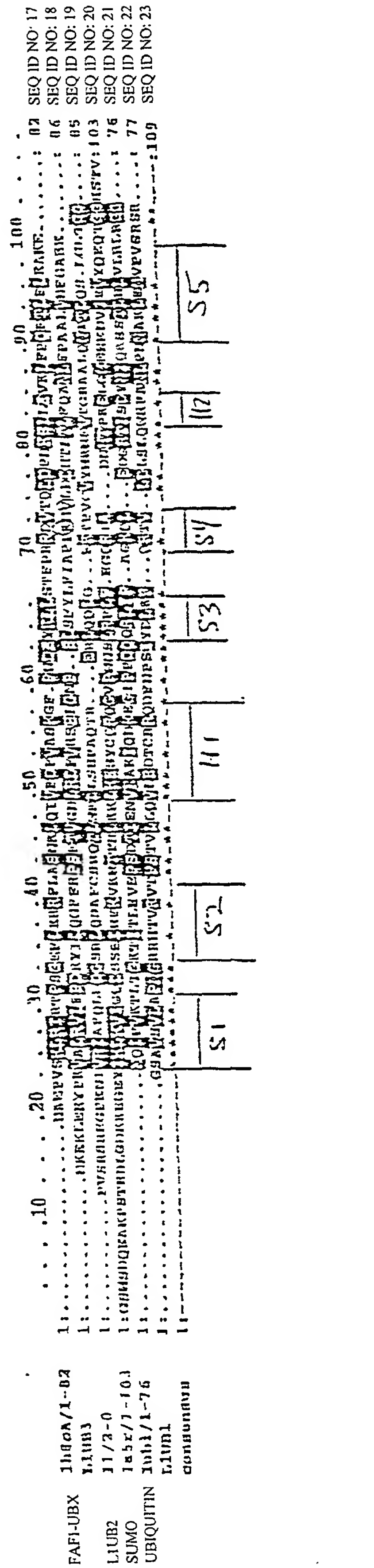
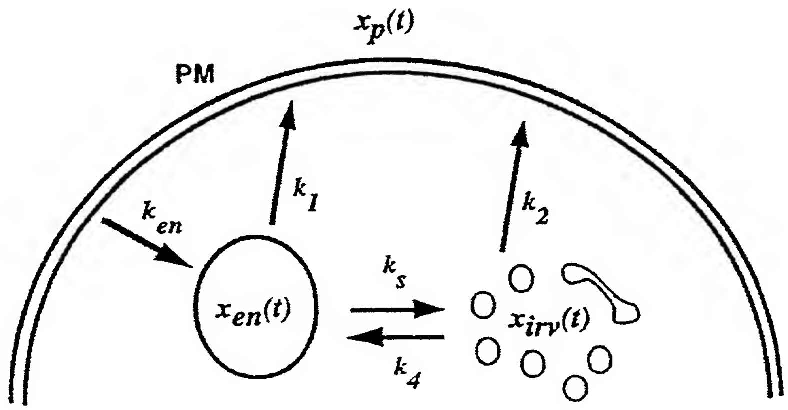

FAF1-UBX
L1UB2
SUMO
UBIQUITIN
SEQ ID NO: 17
SEQ ID NO: 18
SEQ ID NO: 19
SEQ ID NO: 20
SEQ ID NO: 21
SEQ ID NO: 22
SEQ ID NO: 23
S1
S2
H1
S3
S4
H2
S5

Figure 33



$$dx_p(t)/dt = -k_{en}x_p(t) + k_1x_{en}(t) + k_2x_{irv}(t)$$

$$dx_{en}(t)/dt = k_{en}x_p(t) - k_1x_{en}(t) - k_sx_{en}(t) + k_4x_{irv}(t)$$

$$dx_{irv}(t)/dt = k_sx_{en}(t) - k_4x_{irv}(t) - k_2x_{irv}(t)$$

$$x_p(t) + x_{en}(t) + x_{irv}(t) = 1$$

Figure 34

```
Conf:
Pred:
Pred: CCEEEEEEEECCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
  AA: SATIRFVIKQCDTAGKQESIAVRSKAPGSPVSSLSADQAS
               10        20        30        40

Conf:
Pred:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
  AA: SSTLLPLNSGEFSRGDLNHEGDANTSGTGLEGGPKPTDAQ
               50        60        70        80

Conf:
Pred:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
  AA: TKQSTSEPASAPFVPFSGGGQRLGGPSASLRPLTSPSANS
               90       100       110       120

Conf:
Pred:
Pred: CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCEEECCC
  AA: SKSFSGPGGPSKPKKPKPGEEPQQEPEPPVDRDPVVYHPD
              130       140       150       160

Conf:
Pred:
Pred: HHHHHHHCCCCCCHHHHHHHHHHHHHHHHHHHHHHHHHHC
  AA: LEDLLQPWPAEVPDEFFEVTVDDVRRRLAQLKSERKRLEE
              170       180       190       200

Conf:
Pred:
Pred: CCCHHHHHHCCC
  AA: APLVTKAFREAQ
              210
```

Legend:

= helix — Conf: - + = confidence of prediction

= strand — Pred: predicted secondary structure

= coil — AA: target sequence

Figure 35

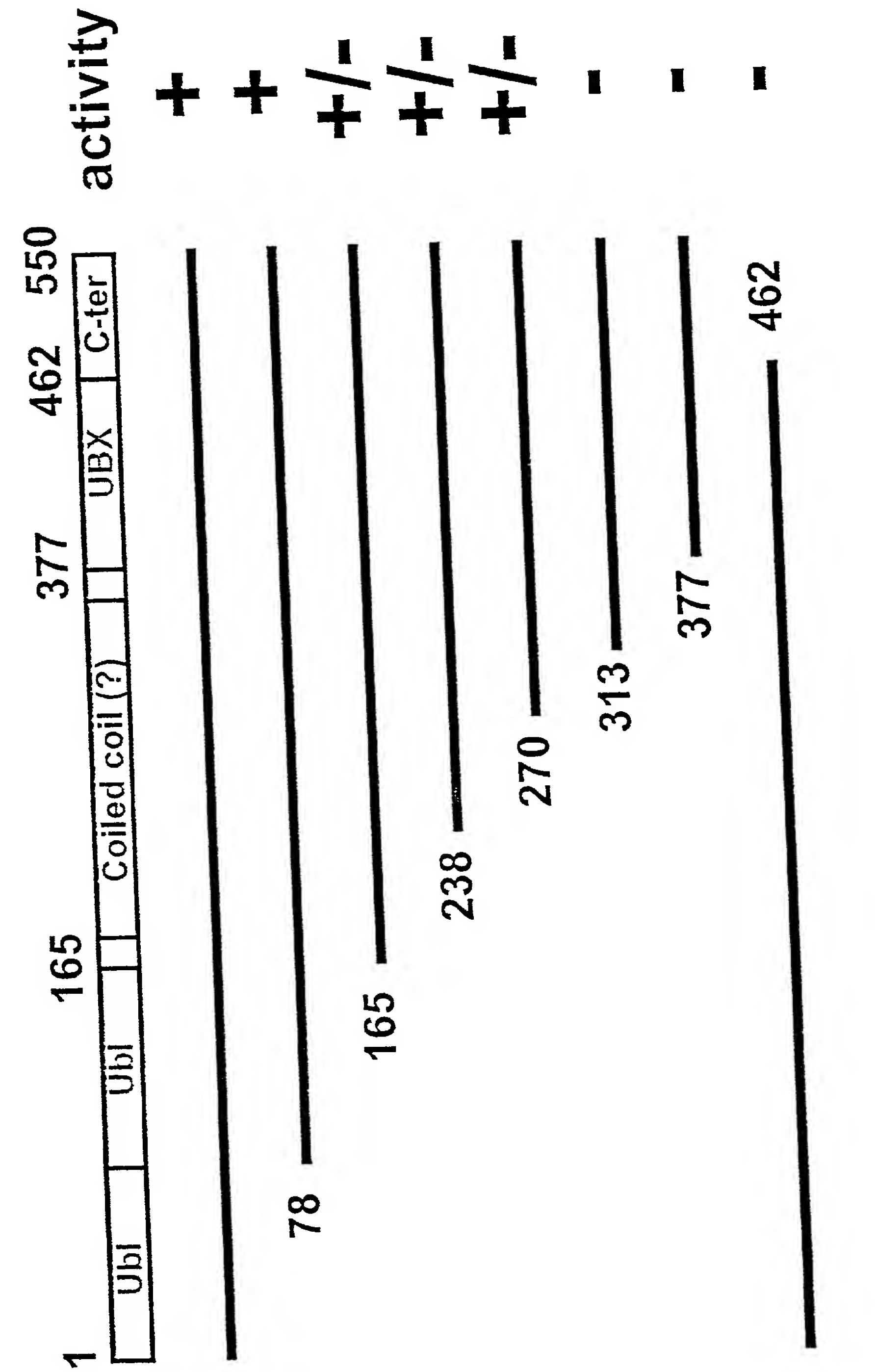

1
165
377
462
550
Ubl
Ubl
Coiled coil (?)
UBX
C-ter
activity
78
165
238
270
313
377
462
+
+
+/-
+/-
+/-
-
-
-

Figure 36